Mechanisms involved in the control of breathing before and after birth

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Voor mijn ouders

Aan mijn grootouders

Contents

Abbreviations

bpm	beats per minute
ECMO	extracorporeal membrane oxygenation
ECoG	electrocortical activity
EMG	electromyographic
HV ECoG	high voltage low frequency electrocortical activity
LV ECoG	low voltage high frequency electrocortical activity
min	minutes
$PaCO_2$	arterial PCO_2
PaO_2	arterial PO_2

Chapter 1

General introduction

In utero, breathing movements are present from early in gestation in animals and man (Connors et al. 1989, Cooke et al. 1990). This fetal breathing activity is similar to respiratory activity after birth since neurons of the respiratory centre the phrenic nerve and the diaphragm are involved (Bahoric & Chernick 1975, Ioffe et al. 1992, de Vries et al. 1982). In utero breathing activity does not accomplish gas exchange because the lungs are filled with fluid and pulmonary circulation is around 15-20% of the right ventricular cardiac output (Iwamoto et al. 1987, Teitel et al. 1990). Then what is the function of fetal breathing activity? It is reported that it is necessary for lung growth and development (Fewell et al. 1981, Liggins et al. 1981a, Liggins et al. 1981b, Harding et al. 1993).

The presence and the regulation of fetal breathing movements has attracted the attention of several groups of investigators. First, acute animal experiments were performed, later chronic animal preparation without the influence of anesthetics allowed more detailed description (Barcroft 1947, Dawes et al. 1972). From those observations the knowledge of regulation of fetal breathing activity increased noticeably but of course many questions remain to be answered, e.g. *a)* what are the stimuli for the presence of fetal breathing movements in utero, *b)* which are the regulators and *c)* what are the changes in the regulation of breathing at birth?

Early in gestation in man breathing activity is present randomly, being a free running activity (Cooke & Berger 1990). In the fetal lamb after maturation of electrocortical activity into LV ECoG and HV ECoG breathing becomes present periodically, only during LV ECoG (Dawes et al. 1972). These observations generated questions on the periodic nature of breathing in utero, its central control and the changes at birth which allow the presence of continuous breathing. It would be interesting to understand fully the mechanisms involved in these changes since it can help to explain some conditions occurring after birth such as apnea of prematurity, sudden infant death syndrome and congenital central hypoventilation syndrome.

The classical challenges to respiratory function after birth, e.g. hypoxemia, hyperoxia, hypercapnia or even hypocapnia, have been studied in the fetal lamb. The expectations were to find similar responses but of course the fetus has different priorities and is in a different environment. This is clearly seen in the response to hypoxemia. In the adult, hypoxemia produces a respiratory stimulation mediated by the peripheral chemoreceptors. However, during hypoxemia fetal breathing activity is inhibited and this is due to central mechanisms (Boddy et al. 1974, Ioffe et al. 1987, Blanco et al. 1983b, Blanco et al. 1984). This response seems appropriate in utero for conservation of oxygen (Rurak & Gruber 1983). To understand better the periodicity of breathing activity it is tempting to speculate that fetal PaO_2, which is approximately 3-4 kPa in late gestation, is sensed as hypoxemia and therefore exerts an inhibitory influence on breathing activity, only overridden by LV ECoG. However, it does not explain why breathing is present before ECoG differentiation and studies of the response range of the peripheral chemoreceptor show that they do not sense the PaO_2 as hypoxia.

Moreover, hyperoxia does not change the incidence of breathing movements in utero (Blanco et al. 1991). This question is still debated (Hasan & Rigaux 1992).

The role of CO_2 in the control of breathing is better understood since the responses are similar to that seen after birth. Hypocapnia is associated with a low incidence of fetal breathing activity (Connors et al. 1988). In utero breathing activity is stimulated by hypercapnia, but it remains present periodically (Boddy et al. 1974, Janssen et al. 1982, Dawes et al. 1982, Ioffe et al. 1987). These observations suggest that the level of $PaCO_2$ plays an important role in the presence of fetal breathing activity. Furthermore, the stimulatory effect of hypercapnia can be an important mechanism in the initiation of continuous breathing at birth (Blanco et al. 1987b).

At the time of birth breathing must become present continuously, i.e. the inhibition which is present in utero must be lifted. There is a long list of factors which facilitate or generate this change, including temperature changes at birth and other afferent inputs such as touch, pain, sound and light, which may be involved in the mechanism for overcoming the central inhibition. When the umbilical cord is clamped, placental circulation is excluded, and therefore possible mediators produced by the placenta which could play a role in fetal breathing regulation, e.g. endorphins, prostaglandins and adenosine, are also excluded. It is known that in utero these mediators can influence fetal breathing activity (Kitterman et al. 1979, Kitterman et al. 1983, Grunstein et al. 1981, Bissonnette et al. 1991).

Many other factors play a role in establishing a new area for gas exchange after cord occlusion. There is a decrease in pulmonary vascular resistance, an increase in pulmonary blood flow, the lungs become expanded with gas etc. The airways are exposed to changes in CO_2 which may influence upper airway receptors (Marsland et al. 1975, Haddad & Mellins 1977, Banzett et al. 1978, Sheldon & Green 1982). However, mechanical ventilation of the lung by itself during normoxic-normocapnia or hyperoxic-normocapnia does not initiate continuous breathing activity in utero (Blanco et al. 1988). Of the many factors which may be involved in the initiation of continuous breathing at birth, in the last few years interest has been focused on hormonal changes after cord occlusion.

The studies of the effects of hypoxemia, hypercapnia or hypocapnia in utero were previously performed by altering maternal blood gases or by reducing uterine blood flow. The exposure of the animal (in this case the ewe) to hypoxemia, hypercapnia or hypocapnia or decreasing uterine blood flow produces a stressful situation causing the release of catecholamines, ACTH and even other substances such as endorphins or prostaglandins, which are known to be respiratory modulators. This is of course a limiting and confounding factor which has to be taken into consideration when interpreting those results. Because of this problem direct access to the fetus was attempted using mechanical ventilation with continuous positive airway pressure (CPAP) or high frequency ventilation oscillation (HFO) but it was difficult to change fetal $PaCO_2$ or PaO_2 independently. Moreover many other receptors could be stimulated when

applying mechanical ventilation or CPAP in utero. We decided to use ECMO as an alternative technique to study fetal breathing and behavioral activity. The ECMO technique is invasive for several reasons. It requires cannulation of vessels such as the jugular vein and carotid artery. The ECMO system requires an extra volume of 350 cc in the circuit compared to the fetal blood volume of approximately 300 cc. The blood is in contact with foreign surfaces (tubing, membrane lung, bladder), therefore activation of inflammatory processes can occur. This of course could introduce confounding variables. It is therefore necessary to control for this using the same criteria as previously to judge physiological behavior, using i.e. ECoG activity, fetal breathing movements, nuchal muscle activity, rapid eye movements and cardiovascular parameters, blood gases and pH (Dawes et al. 1972, Molteni et al. 1980, Clewlow et al. 1983). It is then possible to perform experiments only on fetuses which showed comparable behavioral states, cardiovascular parameters and blood gases and pH as described in the literature. Under these conditions ECMO allowed us to control fetal blood gases, pH and central temperature.

Aims of the thesis

a) Mechanisms involved in the presence of spontaneous breathing activity in utero

It is known that after birth breathing is closely regulated by CO_2 production (Cunningham et al. 1986). The question is whether this mechanism might already be present in utero. Breathing activity is present from very early in gestation. Why this activity is present is not well understood, but it is reasonable to propose that it is dependent on metabolically produced CO_2. This led to the question:

Are breathing movements in utero dependent on the level of $PaCO_2$?

b) Effect of cooling on CO_2 sensitivity

It is well known that fetal breathing activity is stimulated during hypercapnia but it remains periodic, since during HV ECoG breathing activity is inhibited (Boddy et al. 1974, Jansen et al. 1982, Dawes et al. 1982, Ioffe et al. 1987). The periodicity of breathing might be due to a lower sensitivity to CO_2 caused by a lower afferent input to the central nervous system than occurs postnatally. This led to the question:

Does increased afferent input to CNS produced by central cooling change the threshold for CO_2 and override the central inhibition during HV ECoG, resulting in continuous breathing?

c) Analysis of mechanisms involved in the fetal response to hypoxemia

It is known that after birth hypoxemia stimulates breathing activity; in contrast, fetal breathing movements are inhibited during hypoxemia (Boddy et al. 1974, Bryan et al. 1986). One hypotheses is that the fall in PaO_2 is sensed by a central structure, a 'chemoreceptor', located in the brain stem which exerts an inhibitory response (Dawes et al. 1983). Another possibility is that O_2-deficient tissues, including the placenta, release substances such as adenosine, endorphins and prostaglandins which inhibit fetal breathing activity. Previously all the experiments had been done by giving the ewe a hypoxic gas mixture to breathe or by reducing uterine blood flow making the placenta and possibly the ewe hypoxic as well as the fetus. This led to the question:

Are the inhibitory effects on fetal breathing during fetal hypoxemia an indirect effect due to release or production of mediators from the maternal side of the placenta or the ewe?

d) Mechanisms involved in the switch from periodic fetal breathing to neonatal continuous breathing activity at birth

After cord occlusion at birth the inhibition of breathing activity during HV ECoG must be overridden and breathing must become continuous. The onset of continuous breathing has been related to many factors. One of them is temperature since peripheral cooling in utero and after birth resulted in continuous breathing activity (Harned & Ferreiro 1973, Gluckman et al 1983). It was also proposed that the disappearance of an inhibitory substance of placental origin could play a role since breathing activity becomes present continuously after cord occlusion and it is inhibited again after cord release (Adamson et al. 1987, Blanco et al. 1987b). However, in those experiments fetal $PaCO_2$ was not controlled resulting in hypercapnia. The reason for the initiation of continuous breathing after cord clamping in those experiments was thus not clear because the exclusion of a possible placental factor and hypercapnia occurred simultaneously. Thus the questions remained:

Does the exclusion of the umbilical circulation and therefore placental modulators play a role in the initiation of breathing at birth? Does a rise in $PaCO_2$ and changes in temperature play a role during this transition?

Chapter 2

Review of the literature

2.1 Function of fetal breathing movements

Fetal breathing movements are associated with phrenic nerve activity and contraction of the diaphragm (Bahoric & Chernick 1975). It is believed that breathing activity could play a role in lung growth and development. The role of fetal breathing movements on lung growth and development has been studied by diminishing or abolishing breathing activity by spinal cord transections (Liggins et al. 1981a, Harding et al. 1993) or phrenectomy (Alcorn et al. 1980, Fewell et al. 1981). Other investigators increased the compliance of the thorax in order to reduce the negative intrathoracic pressure generated by breathing activity (Liggins et al. 1981b). Lung growth and development were shown to be influenced by these three experimental conditions since the lungs were smaller, had lower DNA content and the distensibility of the fetal lungs decreased (Wigglesworth et al. 1979, Alcorn et al. 1980, Fewell et al. 1981, Liggins et al. 1981a, Liggins et al. 1981b, Bamford et al. 1992, Harding et al. 1993). However, Sival et al. (1992) reported no correlation between the incidence of fetal breathing movements and lung development. Bamford et al. (1992) reported that, despite a small lung, no change in collagen, elastin or DNA content was found.

Lung growth and development are also influenced by altering lung liquid volume. Drainage or ligation of the trachea for 3 to 4 weeks resulted in pulmonary hypoplasia (Alcorn et al. 1977) or an increase in pulmonary lung growth and pulmonary hyperplasia respectively (Alcorn et al. 1977, Wigglesworth et al. 1979). The fall in lung liquid volume after spinal cord transection supports the view that fetal breathing activity stimulates lung growth by its positive influence on lung liquid volume (Dickson & Harding 1991, Fisk et al. 1991, Harding et al. 1993).

In summary, it seems that the presence of a normal incidence of fetal breathing movements is necessary for normal lung growth and development. The mechanism by which fetal breathing activity influences lung growth and development is likely to require continued and controlled release of lung liquid volume.

2.2 Initiation and development

Breathing movements are present in utero from early in gestation. Barcroft (1947)[1] described a spasm of the diaphragm at the 38th day of gestation (term 147 days) in the fetal lamb followed by the first rhythmic movements of the diaphragm at the 40th day of gestation. The earliest chronic recordings from fetal lambs in utero were performed at approximately 50 days of gestation. Two types of diaphragmatic activity could be seen: 1) unpatterned discharge, 2) patterned bursting discharge (Cooke & Berger 1990). Between 75 days and 120 days of gestation, movements of the diaphragm were present periodically, being mainly

associated with nuchal and lateral rectus muscle activity, but there was no relationship with nuchal muscle activity or rapid eye movements (Clewlow et al. 1983, Ioffe et al. 1987). From 108-120 days of gestation breathing movements become more associated with rapid eye movements and increased nuchal muscle activity (Bowes et al. 1981a, Clewlow et al. 1983, Ioffe et al. 1987, Szeto et al. 1992). Electrocortical activity is still undifferentiated and at around 116 days of gestation the frequency was low, approximately 8-16 Hz (Szeto 1990).

In the human fetus breathing movements start to occur between the 10-12th week of gestation (de Vries et al. 1982).

In summary, breathing activity is present from early in gestation in animals and man. Breathing movements are present irregularly without a clear pattern which suggest a nonregulated activity.

2.3 Central control

2.3.1 Electrocortical activity

In the fetal lamb the most important change in the organization of breathing and behavioral activity occurs after maturation of the forebrain. By 120-125 days of gestation electrocortical activity matures into clearly identifiable states, LV ECoG, with a frequency of 13-30 Hz, and HV ECoG, with a frequency of 3-9 Hz (Dawes et al. 1972, Clewlow et al. 1983, Szeto et al. 1985, Szeto 1990). In fetal lambs electrocortical activity alternates between LV ECoG and HV ECoG after this point in gestation.

After maturation of electrocortical activity fetal breathing movements become present periodically, occurring only during 40% of the total time and only during LV ECoG. These fetal breathing movements are rapid and irregular, have a frequency of 0.1-4 Hz, an amplitude of 3-5 mmHg and produce only small changes in lung liquid volume (<1ml) (Harding et al. 1984).

During LV ECoG fetal breathing movements are present for 70% of the time, being associated with rapid eye movements. During LV ECoG there is an inhibition of postural muscle tone (Dawes et al. 1980, Ioffe et al. 1980) and the amplitude of spinal polysynaptic reflexes is reduced (Blanco et al. 1983b). This LV ECoG state is comparable to REM sleep after birth (Phillipson & Bowes 1986). LV ECoG activity is associated with a significantly lower heart rate, a decrease in blood pressure, a decrease in placental blood flow and an increase in central blood flow (Clapp et al. 1980, Richardson et al. 1985, Abrams et al. 1991). Sleep state modulation of blood pressure and heart rate is independent of intact vagi and/or carotid sinus nerves (Koos & Sameshima 1988c). The irregularities in heart rate variability during LV ECoG may be caused by the fall in intrathoracic pressures associated with respiratory movements (Dawes 1973).

During HV ECoG breathing movements are inhibited (Dawes et al. 1983, Gluckman & Johnston 1987, Johnston & Gluckman 1989). HV ECoG is associated with sustained contractions of nuchal muscles, there is spontaneous gastrocnemius activity and the magnitude of spinal monosynaptic and polysynaptic reflexes are present (Dawes et al. 1972, Ruckebusch et al. 1977, Natale et al. 1981, Rigatto et al. 1982, Clewlow et al. 1983, Ioffe et al. 1987). It is not known why fetal breathing movements are inhibited during HV ECoG and how this mechanism is overridden during LV ECoG.

Different experiments lead to the conclusion that the inhibition of fetal breathing movements during HV ECoG is of central origin (Dawes et al. 1983, Johnston & Gluckman 1989). Breathing movements were dissociated from ECoG after transection at the level of the colliculi (Dawes et al. 1983) or after bilateral lesions in the lateral pons, placed stereotaxically (Gluckman & Johnston 1987, Johnston & Gluckman 1989). Furthermore, it has been suggested that during HV ECoG is an increase in the threshold for breathing. This was illustrated in experiments using fetal lambs with lesions in the lateral pons in the lateral parabrachial/Kolliker - Füse region. In these fetuses breathing activity was present periodically during normocapnia, but during lactic acidosis or hypercapnia breathing activity became present continuously (Johnston & Gluckman 1983).

So far we have described 2 sleep states, but it is also possible that wakefulness is present in utero and breathing activity could be regulated differently in that state. Fetal wakefulness was defined by the simultaneous presence of LV ECoG, rapid eye movements and neck muscle tone, being present only 5-6% of the time (Ioffe et al. 1980, Rigatto et al. 1982). However, observations through a Plexiglas window implanted in the flank of the ewe did not confirm the occurrence of wakefulness as defined postnatally (Rigatto et al. 1986).

In the fetal lamb during the last trimester of gestation, breathing movements may be part of the behavioral expression of LV ECoG since they are only present during this state. Behavioral states are constellations of physiological and behavioral variables which are stable over time and recur repeatedly, not only in the same fetus but also in similar forms in all fetuses at a similar age (Prechtl et al. 1968). In utero HV ECoG is associated with nuchal muscle activity, absence of rapid eye movements and absence of breathing movements, and LV ECoG is associated with absence of nuchal muscle activity, the presence of rapid eye movements and the presence of breathing movements (Richardson et al. 1985, Bocking & Harding 1986).

In the human, fetal breathing movements are also present periodically. It was reported that rapid eye movements and the presence of breathing movements were highly correlated after 27 weeks of gestation (Okai et al. 1992). Although fetal breathing movements did not contribute to the definition of fetal behavioral states, the regularity and frequency of fetal breathing movements were influenced by states (Nijhuis et al. 1982, Arabin & Riedewald 1992, Mulder et al. 1994). Behavioral states in the human fetus were defined by fetal body movements, rapid eye movements

and heart rate pattern. The coordination among these variables are already present around 32 weeks of gestation (Visser et al. 1987). By 38 to 40 weeks of gestation 4 distinct fetal states were present equivalent to 4 of the 5 states described in the newborn (Nijhuis et al. 1982).

In summary, electrocortical activity differentiates into two states in fetal lambs at 120-125 days of GA. The presence of breathing activity is state dependent since breathing movements are only present during LV ECoG. During HV ECoG breathing movements are inhibited, this inhibition being of central origin. Breathing activity early in gestation seems to lack pattern, therefore it is difficult to explain its presence as a behavioral expression. In the human fetus breathing movements are periodically present but they do not contribute to the definition of fetal behavioral states since they are not limited to a definite state.

2.4 Metabolic control

2.4.1 Glucose

In the fetal lamb, as in the human, glucose is the major metabolic substrate. Fetal breathing movements are likewise influenced by circulating levels of glucose.

In the fetal lamb maternal hypoglycemia due to lack of food intake is associated with a reduction in the incidence of LV ECoG and fetal breathing movements. Infusion of glucose to fasted fetuses resulted in an increase of breathing movements to a level similar to the control values (Richardson et al. 1982).

In the human fetus, the incidence of fetal breathing movements is also influenced by glucose. Breathing movements were stimulated by hyperglycemia (Natale et al. 1978, Patrick et al. 1978, Bocking et al. 1982). The stimulation of breathing activity during hyperglycemia might be explained by the oxidation of glucose to CO_2 and H_2O. These increased concentrations of CO_2 could stimulate respiratory activity (Natale et al. 1978, Bocking et al. 1982, Richardson et al. 1983). Of course until recently fetal blood gases and pH could only be measured in fetal sheep (Bocking et al. 1982, Richardson et al. 1983). Another possibility might be that glucose increases the incidence of LV ECoG (Richardson et al. 1982)

In summary, glucose levels influence fetal breathing activity.

2.4.2 Carbon dioxide

CO_2 is an end product of metabolism and is directly related to O_2 consumption under aerobic conditions. In the adult the elimination of CO_2 is achieved by two mechanisms, a fast mechanism by pulmonary

ventilation and a slow mechanism by renal excretion as bicarbonate. Therefore, there is a very close relationship of alveolar ventilation to CO_2 production (Cunningham et al. 1986).

2.4.2.1 Hypocapnia

In the adult CO_2 production and its oscillations play an important role in the control of breathing (Cunningham et al. 1986, Eldridge & Millhorn 1986). The maintenance of breathing activity is critically dependent on the level of $PaCO_2$ during all stages of sleep. Mild hypocapnia ($P_{end\ tidal}CO_2 < 4.6$ kPa) leads to apnea, except during wakefulness when behavioral drives supervene (Datta et al. 1991, Canet et al. 1993). During the first two weeks of life the apnea threshold was 1-1.2 kPa lower than immediately after birth which indicated resetting of this threshold (Canet et al. 1993). Thus breathing activity is modulated by $PaCO_2$.

Whether the presence of fetal breathing activity is determined by $PaCO_2$ is still unsettled. In utero breathing activity does not provide any gas exchange since this is provided by the placenta. Therefore, it would not be logical to expect that CO_2 production or $PaCO_2$ level would control breathing activity in utero as postnatally.

To our knowledge no conclusive study of the effect of fetal hypocapnia on fetal breathing activity in the fetal lamb has been reported. Boddy et al. (1974) described 2 fetal lambs that became hypocapnic and remained normoxic spontaneously due to hyperventilation of the ewe. These fetuses showed a decreased incidence (15%) of fetal breathing activity but the duration of this effect was not reported. After administration of 3% of CO_2 to the mother breathing activity returned to its normal incidence.

In the human fetus a decrease in breathing movements has been produced during hypocapnia obtained by maternal hyperventilation in the last weeks of gestation (Marsál et al. 1979, van Weering et al. 1979, Connors et al. 1988). These experiments were limited to a maximum of 15 min and no fetal blood gases were obtained.

In the human and sheep hypocapnia could cause a decrease in uterine blood flow (Leduc 1972, Levinson et al. 1974, Oakes et al. 1976), an increase in placental vascular resistance (Motoyama et al. 1967, Oakes et al. 1976) and a decrease in umbilical blood flow (Motoyama et al. 1967) possibly resulting in hypoxemia. Therefore, the mechanism responsible for the decrease in fetal breathing movements during hypocapnia in those experiments is not clear since hypocapnia and hypoxemia may have occurred simultaneously (Marsál et al. 1979) and hypoxemia is a known inhibitor of fetal breathing movements (Boddy et al. 1974).

One of the problems of fetal hypocapnia obtained by any of the methods is that it can produce a decrease in cerebral blood flow which may result in central nervous system hypoxemia and this may have been responsible for the decrease in breathing activity during hypocapnia. However, in piglets hypocapnia was associated with a significant decrease of cerebral blood flow only when $PaCO_2$ decreased to extreme degrees

($PaCO_2$ < 2 kPa) and blood flow to the brain stem, thalamus and cerebellum was preserved (Hansen et al. 1984).

In summary, after birth CO_2 level influences breathing activity but in utero this is less clear. Hypocapnia experiments were performed by maternal hyperventilation which might have resulted in hypoxemia due to a decrease of uterine blood flow, an increase in placental vascular resistance and a decrease of umbilical blood flow. Furthermore, these experiments were limited in duration. Thus whether the incidence of fetal breathing movements are influenced by hypocapnia remains unclear.

2.4.2.2 Hypercapnia

Hypercapnia is a well known respiratory stimulant which produces its effect at peripheral and central chemoreceptors. The effects are expressed as a stimulation of ventilation which is clearly directed to decrease $PaCO_2$. What is the situation in utero?

From 0.5 of gestation in the fetal lamb hypercapnia causes a change from irregular tonic to regular phasic activity in diaphragmatic EMG activity already (Ioffe et al. 1987). In the human fetus hypercapnia stimulates breathing activity from 24 weeks' of gestation (Connors et al. 1989).

During hypercapnia in fetal lambs, after maturation of electrocortical activity, there is an increase in incidence of breathing activity and it becomes deeper and more regular (Boddy et al. 1974, Chapman et al. 1980, Dawes et al. 1982). Hypercapnia is not sufficient to produce continuous breathing since breathing activity remains associated with LV ECoG and is inhibited during HV ECoG (Jansen et al. 1982, Rigatto et al. 1988). Therefore, the increase in incidence of breathing activity appears to be due to a change in behavioral activity since there is a significant increase in incidence of LV ECoG, the state in which fetal breathing activity occurs (Boddy et al. 1974). An increase in breathing activity in hypercapnia is also described in human fetuses (Ritchie & Lahkani 1980, Connors et al. 1988, Connors et al. 1989). However, in the human fetus there is no change in behavioral activity during hypercapnia, which suggests that in the human fetus hypercapnia stimulates the respiratory centre directly rather than via a change in behavioral activity (O'Grady et al. 1983).

The mechanisms involved in the stimulation of breathing during hypercapnia are not known. In utero peripheral chemoreceptors are active and responsive to natural stimuli (Blanco et al. 1984). Injection of CO_2 saturated saline towards the carotid chemoreceptors resulted in an increase in their firing activity. However, after carotid denervation and vagal section the incidence of fetal breathing movements still increased significantly during hypercapnia (Koos & Sameshima 1988c). Therefore, it has been suggested that the response is mediated by the central chemoreceptors (Dawes et al. 1982, Jansen et al. 1982, Koos et al. 1988c). An elevation of $PaCO_2$ alone is not sufficient to produce

breathing in fetal lambs during HV ECoG (Dawes et al. 1982, Jansen et al. 1982, Rigatto et al. 1988). This suggests that during HV ECoG there is a higher threshold for stimulation of breathing, due to an inhibition which is lifted during LV ECoG. The response to hypercapnia was similar in intact fetal lambs and in caudal brain stem sectioned fetal lambs. In rostral brain stem sectioned fetal lambs the response to CO_2 was greater than in intact lambs. This suggests that a degree of control by higher centres operates normally in intact animals (Dawes et al. 1983). This was further studied in fetal lambs with lesions in the rostral lateral pons. In those animals breathing activity remained associated with LV ECoG during normocapnia. Hypercapnia produced an increase in incidence of breathing, and breathing activity became continuous in 4 fetal lambs and almost continuous in another 3 fetal lambs (Johnston & Gluckman 1989). These results suggest that there is a central regulation of the CO_2 sensitivity in utero which is dependent on a small area in the rostral lateral pons (nucleus or pathway).

In utero CO_2 is a potent stimulus of breathing activity which is convenient since hypercapnia plays an important role in the initiation of continuous breathing at birth (Blanco et al. 1987b). At the time of birth there is an increase in afferent input from cooling, touch, pain, sound and light and these stimuli can generate or facilitate the drive to breathe (Condorelli & Scarpelli 1975, Scarpelli et al. 1977, Gluckman et al. 1983). Indirect evidence supporting this was reported by Moss & Scarpelli since the threshold for CO_2 was lowered in acute experiments in fetal lambs by sciatic nerve stimulation or cooling (Moss & Scarpelli 1979, Moss et al. 1983). Furthermore, at the time of birth cold could override the negative effects of hypocapnia (Blanco et al. 1987b). This could lead to the idea that extra afferent input might change the sensitivity for CO_2 resulting in the initiation of continuous breathing at birth.

In most experiments fetal hypercapnia was obtained by producing maternal hypercapnia. Therefore, the fetal responses were not independent of maternal influences. Maternal hypercapnia can result from a stressful situation which can cause an increase in fetal plasma epinephrine (Faucher et al. 1991), maternal and fetal cardiovascular changes, such as hypertension and tachycardia, consistent with sympathetic activation (Walker et al. 1976).

In summary, hypercapnia stimulates fetal breathing activity but it remains present periodically. It may be speculated that extra afferent input might change the sensitivity to CO_2, contributing to the initiation of continuous breathing at birth. So far fetal hypercapnia experiments have been obtained by maternal hypercapnia which limits interpretation and introduces confounding variables.

2.4.3 Oxygenation

Fetal PaO_2 is low (approximately 3 kPa) but the oxygen supply is twice the value necessary to maintain normal O_2 consumption with aerobic metabolism (Wilkening & Meschia 1983). This level of oxygenation allows the presence of normal behavior, fetal growth and development. Nevertheless, fetal breathing movements are present periodically and hypoxemia exerts a centrally-mediated inhibitory influence (Dawes et al. 1983). It has been suggested that fetal normoxemia might be sensed as hypoxemia resulting in an inhibition which might cause periodicity of fetal breathing. The presence of fetal breathing movements during LV ECoG can be seen as a lifting of the inhibition by this state. Furthermore, the fact that at birth breathing becomes continuous through all states may be interpreted as a lifting of this tonic inhibitory influence by the increase in PaO_2 which follows birth. The question is whether this mechanism would explain initiation of breathing after cord occlusion at birth. It is highly unlikely that PaO_2 plays an important role in the initiation of breathing activity at birth since PaO_2 at birth is at fetal level or even lower (Berger et al. 1990) but oxygenation after birth is essential for the maintenance of breathing.

In summary, oxygenation might play a role in determining the periodicity of fetal breathing activity.

2.4.3.1 Hypoxemia

After birth, hypoxemia produces a stimulatory effect, there being an increase in ventilation, arousal and persistence of spinal reflexes (Blanco et al. 1983a, Cunningham et al. 1986, Phillipson & Bowes 1986). This response seems to be mostly mediated by the peripheral chemoreceptors since after chemodenervation the effect of hypoxemia tends to be inhibitory.

In utero, the response to hypoxemia is different, as it causes inhibition. This inhibitory fetal response is present within 5 min of exposing the ewe to severe hypoxemia (9% O_2, 3% CO_2 and 88% N_2) (Boddy et al. 1974, Koos et al. 1987a). This overall inhibition of fetal activity is represented by a decrease of limb movements, nuchal muscle activity (Natale et al. 1981, Bocking & Harding 1986, Martin et al. 1987, Woudstra et al. 1990), rapid eye movements (Boddy et al. 1974, Natale et al. 1981, Bocking & Harding 1986, Koos et al. 1987a), fetal swallowing (Sherman et al. 1991) and a reduction of the magnitude of hind-limb reflexes (Blanco et al. 1983b). The effect of hypoxemia on ECoG activity is controversial as the incidence of LV ECoG activity is reported to decrease (Boddy et al. 1974, Natale et al. 1981, Bocking & Harding 1986) or remain unchanged (Adamson et al. 1984, Koos et al. 1987a, Bocking et al. 1988). The number of switches from HV ECoG to LV ECoG activity is reported to increase (Boddy et al. 1974, Clewlow et

al. 1983, Koos et al. 1987a) or to remain unchanged (Adamson et al. 1984, Bocking & Harding 1986). Therefore, during hypoxemia ECoG activity may or may not change. However, the usual associations within states are missing, for instance during HV ECoG there is an inhibition of reflexes and of nuchal muscle activity (Blanco et al. 1983b, Woudstra et al. 1990). Furthermore, LV ECoG state is also not complete since there are no breathing movements and rapid eye movements present.

The mechanisms regulating fetal breathing movements, rapid eye movements or spinal reflexes seem to be different since during a prolonged hypoxemia period (48 hours) rapid eye movements were present at the end of the period but breathing movements were inhibited (Bocking et al. 1988). Also the mechanisms involved in the inhibition of spinal reflexes and breathing movements are different. In fetal lambs transected at the level of the colliculli hypoxemia stimulated breathing movements but spinal reflexes were inhibited (Blanco et al. 1983b).

The decrease of fetal breathing movements and muscle activity can be considered a logical fetal response since fetal breathing movements and muscle movements are associated with increased oxygen consumption (Rurak & Gruber 1983). It is reported that oxygen consumption is diminished during hypoxemia (Parer 1980).

This inhibitory response exists already at 0.5 of gestation (Clewlow et al. 1983, Ioffe et al. 1987). However, fetal breathing activity is less affected in early gestation than in late gestation (Clewlow et al. 1983, Bocking & Harding 1986).

Several methods have been used to study the effect of hypoxemia on fetal activity. The two most common methods for reducing fetal PaO_2 are occlusion of the maternal common internal iliac artery or maternal hypoxia induced by making the ewe breathe a hypoxic gas mixture. Other possibilities for inducing fetal hypoxemia are decreasing O_2 carrying capacity of the fetal blood with a decrease in viscosity (fetal anemia), by decreasing O_2 carrying capacity of the fetal blood without a decrease in viscosity (fetal methemoglobinemia) or by changing oxygen affinity (carboxyhemoglobinemia) (Koos et al. 1987b, Koos et al. 1988b, Koos et al. 1990a). Hypoxemia obtained by any of these methods results in a decrease of fetal breathing movements and rapid eye movements (Boddy et al. 1974, Bocking & Harding 1986, Bocking et al. 1988, Koos et al. 1988a, Koos et al. 1990a). Since anemia resulted in decreased oxygen content without changing PaO_2, this might imply that inhibition is triggered by low tissue oxygenation rather than actual values of PaO_2 (Koos et al. 1987b).

The inhibitory effects on fetal breathing movements produced by hypoxemia or acute anemia were transient since the incidence of breathing activity returned to control within 14-16 hours after initiation of hypoxemia or hypocapnic hypoxemia (Bocking et al. 1988, Koos et al. 1988a) or within 2-3 hours during anemia (Bissonnette & Hohimer 1987, Matsuda et al. 1992). The mechanisms involved in this adaptation are unclear (Hohimer & Bissonnette 1991).

The mechanisms involved in the fetal inhibitory response to hypoxemia are not yet clear. The inhibition of fetal breathing movements during

hypoxemia seemed not to be due to lack of peripheral chemoreceptor input since carotid and aortic chemoreceptor afferent discharge activity increased as PaO_2 decreased (Blanco et al. 1984). Therefore, the lack of an stimulatory response is not due to diminished input but to an increased threshold for responding to this activity.

Evidence against a non-specific direct hypoxic depression came from work of Barcroft (1947) and Dawes et al. (1983) where the inhibitory effect of hypoxemia on breathing movements in fetal sheep was abolished by brain stem transection at the level of the colliculi (at the pontine-midbrain junction) (Dawes et al. 1983). Gluckman and Johnston reported similar observations after producing lesions within the pons in the region of the lateral parabrachial and Kolliker-Füse nuclei close to the trigeminal motor nuclei (Gluckman & Johnston 1987, Johnston & Gluckman 1989). In these preparations breathing activity was present periodically but was stimulated during hypoxemia. The hypothesis that an area in the pons could function as a sensor for hypoxemia was further investigated in kittens and newborn lambs. Stimulation in the rostral ventral pons resulted in an inhibition of breathing activity (Coles et al. 1989, Noble & Smith 1991) and the discharge frequencies of neurones in this area increased significantly during hypoxemia (Noble & Williams 1989). This supports the view that the response to hypoxemia is mediated or integrated within the pons.

It is known that hypoxia reduces mitochondrial ATP production and this could be the mechanism by which hypoxemia is sensed by the putative 'pontine chemoreceptor' resulting in inhibition of fetal breathing activity. Evidence for this was suggested by experiments using Oligomycin B (inhibitor of mitochondrial ATPase) where the incidence of breathing movements was decreased without changes in ECoG activity or rapid eye movements (Koos et al. 1986). The experiments described tend to support the idea that tissue PO_2 or oxygenation is the stimulus to trigger this fetal inhibition. It is possible that other mechanisms or stimuli are involved in this response still using this pontine structure or pathway as a component in the response.

During hypoxemia, O_2 deficient tissues, including the placenta, are likely to release adenosine, endorphins, prostaglandins etc. These substances can influence fetal breathing movements, other muscle activities and ECoG activity.

It was suggested that adenosine played a role in modulating fetal breathing activity during normoxia and hypoxemia in fetal sheep (Bissonnette et al. 1990, Koos et al. 1990b). During hypoxemia, due to a decrease in oxidative phosphorylation, adenosine increased centrally or peripherally (Koos et al. 1990b, Koos & Doany 1991). It is known that placental tissue produces adenosine during hypoxemia (Slegel et al. 1988). The effects of adenosine are very similar to the effects of hypoxemia, both before and after brain stem transection in fetal lambs (Dawes et al. 1983, Koos et al. 1992). The intravenous infusion of an adenosine analogue in intact fetal lambs produced a temporary reduction of breathing and rapid eye movements but after brain stem transection breathing activity increased. Therefore, it was suggested that adenosine

played an important role in producing the hypoxemic response and that it could be involved in the transmission of this inhibition locally (Koos et al. 1990b, Koos & Doany 1991). Adenosine antagonists, e.g. theophylline, stimulated breathing movements during normoxia (Bissonnette et al. 1990, Bissonnette et al. 1991) and prevented the inhibition during hypoxemia (Koos et al. 1990b).

Endorphins are elevated during hypoxemia in fetal lambs (Wardlaw et al. 1981). In these experiments, in which the ewe was breathing a hypoxic gas mixture, maternal beta-endorphin immunoactivity were not changed which indicated that the increase in fetal beta-endorphin was not of maternal origin. This rise in fetal endorphins might be due to an increase in the secretion of endorphins from the fetal pituitary, the placenta or to decreased degradation of endorphins. It might be speculated that the increased levels of endorphins play a role in the inhibition of fetal breathing activity. It was also reported that endorphins were involved in the pathogenesis of apnea in the newborn (Chernick et al. 1981, Grunstein et al. 1981, McQueen 1983, Santiago & Edelman 1985).

PGE_2 is known to inhibit fetal breathing movements (Kitterman et al. 1983). However, during hypoxemia serum PGE_2 levels were only significantly increased after 4 hours of hypoxemia and remained elevated for 24 hours (Akagi et al. 1990a, Akagi & Challis 1990b, Sue-Tang et al. 1990). Therefore, a role of PGE_2 as mediator for the inhibitory response appears unlikely since during hypoxemia breathing movements are inhibited within 5 min and return to baseline within 16 hours (Boddy et al. 1974, Bocking et al. 1988, Koos et al. 1988a).

During hypoxemia there is a significant increase in norepinephrine and epinephrine (Lewis et al. 1982). However, the effect of catecholamines on breathing activity remains unsettled since the incidence of breathing activity decreased, increased or did not change during normoxia (Murata et al. 1981, Cohen et al. 1982, Jansen et al. 1986). During hypoxemia infusion of a beta-agonist resulted in a decrease in breathing activity (Jansen et al. 1986). Infusion of an alpha-antagonist resulted in the presence of breathing activity even during hypoxemia (Bamford & Dawes 1990, Giussani et al. 1992). It has been suggested that alpha-adrenergic mechnisms have an inhibitory influence on fetal breathing movements even during normoxia and hypoxia.

After infusion of modulators or drugs (660 ug morphine, meclofenamate, corticotrophin releasing hormone, thyrotropin releasing hormone, 5-HTP, GABA-antagonist picrotoxin) breathing activity may either increase or changed from intermittent to continuous breathing activity. The tonic inhibition or threshold during HV ECoG can be lifted by different mechanisms, however, the inhibitory effects of hypoxemia are still present (Quilligan et al. 1981, Johnston & Gluckman 1983, Koos 1985, Bennet et al. 1988, Bennet et al. 1990b).

In summary, the fetal response to hypoxemia before birth is clearly inhibitory in contrast to that after birth. Stimuli for this response are a decrease in tissue oxygenation by low PaO_2 or oxygen content. The mechanisms involved in this response seem to be integrated or mediated

in the brain stem. Neurotransmitters and hormonal changes could play a role but this is not very well understood.

2.4.3.2 Hyperoxia

In the adult hyperoxia can result in transient changes in breathing activity within seconds by silencing the spontaneous activity of peripheral chemoreceptors. This relatively small effect of hyperoxia on the control of breathing appears to be a natural consequence of the only minor impact of hyperoxia on tissue oxygenation (Dejours 1962). The situation in the fetus is different since the fetus is chronically exposed to a low PaO_2 in comparison to postnatal values. This physiological low PaO_2 could be sensed as hypoxemia and exert a central tonic inhibition, only lifted during LV ECoG. Therefore, it could be attractive to think that this inhibition could be lifted at birth allowing continuous breathing to occur.

In chronically instrumented fetal lambs, raising fetal PaO_2 to neonatal levels (8-12 kPa) using ECMO failed to induce continuous breathing activity (Blanco et al. 1991) nor did continuous breathing occur, when fetal PaO_2 was raised by expanding the lungs using mechanical ventilation (Blanco et al. 1987a). These results were consistent with the results of Hasan et al. (1991) who reported no change in breathing activity in fetuses with a gestational age < 134 days. However, in older fetuses it was reported that breathing activity became continuous in one of the six fetuses of 135 ± 1 days gestation at a PaO_2 of 8.8 kPa and in four of the six fetuses of 138 ± 1 days of gestation. In these latter fetuses the PaO_2 increased to a mean of 32 kPa. In a later study Hasan et al. (1992) reported that fetal breathing activity became present continuously and was associated with arousal (defined by LV ECoG and the presence of nuchal muscle activity and rapid eye movements). In seven of the ten fetuses (135-142 days of gestation) PaO_2 was approximately 15 kPa. In these experiments hyperoxia was obtained using CPAP to a pressure of up to 40 cm H_2O (Hasan et al. 1991, Hasan et al. 1992). The effect of the lung distension on fetal cardiovascular and fetoplacental blood flow induced by these levels of CPAP is not known. Towards term the effect of the increase in intrathoracic pressure induced by CPAP would be more profound in the relatively mature fetuses due to a rise in lung compliance. Confounding variables induced by these levels of CPAP cannot be excluded. Baier et al. (1990, 1992a, 1992b) reported that during hyperoxia breathing activity also became present continuously in association with cord clamping, but again the levels of fetal PaO_2 were unphysiologically high and occurred most of the time with a significant increase of $PaCO_2$ and decrease in pH. Other mechanisms could have been involved in producing continuous breathing activity, e.g. a significant decrease in cerebral blood flow occurred when fetal PaO_2 was between 10-25 kPa (Blanco et al. 1988) resulting in a rise in $PaCO_2$ in the environment of the medullary chemoreceptors. So far, the presence of continuous breathing activity in utero during hyperoxia was mainly associated with an unphysiological increase of PaO_2, an increase in $PaCO_2$ and a decrease in

pH. Only in fetuses older than 134 days gestation did breathing become present continuously occasionally, with a physiological PaO_2 but with lung distended at a CPAP around 20-40 cm H_2O. These experiments show that fetal maturity may play a role, but clearly they also raise many other questions.

Maternal hyperoxia can be obtained by the ewe breathing a hyperoxic gas mixture (Khazin et al. 1971, Boddy et al. 1974, Towell et al. 1984). The increase in fetal PaO_2 is not proportional to the increase in maternal PaO_2 (Khazin et al. 1971, Boddy et al. 1974). Hyperbaric oxygenation (Tiktinsky et al. 1992), mechanical ventilation in utero (Blanco et al. 1987a) or connection to an ECMO system (Blanco et al. 1991) are other possibilities for raising fetal PaO_2. During hyperbaric oxygenation $PaCO_2$ increases significantly which has a stimulatory effect on breathing activity. During mechanical ventilation, the lungs are distended and breathing activity may have been influenced by upper airway CO_2 sensitive receptors since CO_2 passes the airway (Marsland et al. 1975, Haddad & Mellins 1977, Banzett et al. 1978, Sheldon & Green 1982).

In summary, in fetuses up to 134 days of gestation, breathing activity does not become present continuously during hyperoxia. In fetuses older than 135 days of gestation the results are still controversial since frequently PaO_2 was unphysiologically high.

2.4.4 Metabolic acidosis

It is well known that in the adult central metabolic acidosis stimulates breathing activity (Pappenheimer et al. 1965). In utero the effect of metabolic acidosis was studied by infusing NH_4Cl, HCl and lactic acid i.v. or by infusing HCl, mock CSF with low $[HCO_3^-]$ and low pH into the ventriculocisternal system.

Metabolic acidemia produced by peripheral infusion of NH_4Cl or HCl resulted in an increase in incidence and depth of breathing movements but breathing seldom became continuous (Molteni et al. 1980, Hohimer & Bissonnette 1981, Koos 1985). These results contrast with those of Johnston and Gluckman (1989) who reported that peripheral infusion of lactic acid resulted in a decrease of breathing activity during LV ECoG.

The effect of central infusion of artificial CSF with low $[HCO_3^-]$ content resulted in an increase in incidence and amplitude of fetal breathing movements and breathing activity became present continuously (Hohimer et al. 1983, Koos 1985, Walker & Davies 1986).

In fetuses with lesions in the rostral lateral pons peripheral infusion of lactic acid resulted in breathing activity both during LV ECoG and HV ECoG (Johnston & Gluckman 1989).

In summary, metabolic acidosis stimulates breathing activity. Continuous breathing activity occurred in only a few fetuses after peripheral infusion of NH_4Cl. Central metabolic acidosis obtained by low $[HCO_3^-]$ overrides the inhibition of HV ECoG and allows continuous breathing activity in

utero. It is believed that the stimulation of this breathing activity is mediated by central chemoreceptors in the medulla.

2.5 Peripheral control

After birth peripheral control is an important component in the control of breathing. These peripheral mechanisms are mainly directed to provide information from the periphery to the CNS about the environment and the function of the lung. In utero oxygenation is provided by the placenta. Therefore, the lungs do not function as a gas exchange area and the fetus is protected from the environment. This provide some circumstantial evidence that peripheral information to the fetal brain is not of importance for the control of this 'fetal respiratory activity'.

2.5.1 Chemoreceptors and lung afferents

At birth, the carotid body chemoreceptors are not essential for the establishment of effective rhythmic breathing (Jansen et al. 1981). In utero, fetal breathing is clearly modulated by central mechanisms as suggested by the changes with ECoG state. Furthermore, chemoreceptors and vagal afferents seemed to be of little influence since vagotomy and carotid chemodenervation did not change the characteristics of fetal breathing activity (Dawes et al. 1972, Boddy et al. 1974). In the fetal lamb vagotomy did not modify the size, frequency or incidence of rapid irregular breathing movements (Boddy et al. 1974) and carotid sinus denervation did not alter the incidence or characteristics of fetal breathing movements (Jansen et al. 1981, Moore et al. 1989). Murai et al. (1985) reported a decrease in incidence and amplitude of breathing movements after bilateral section of both vagus and carotid sinus nerves, although the incidence was normal 5 days after of surgery. This reported decrease might had been a result of surgery. Physiological denervation of the peripheral chemoreceptors by hyperoxia did not change the incidence of fetal breathing activity (Blanco et al. 1991). However, carotid chemoreceptors are active in utero (Blanco et al. 1984, Johnston et al. 1994). It is known that carotid chemoreceptors do have a spontaneous activity and react to an increase in CO_2 and a decrease in O_2. Therefore, there must be a central mechanism which will increase the threshold for receiving and processing this information. Even though pulmonary afferents seem not to be actively involved in the regulation of breathing activity in utero, they can be involved when the fetal lung is inflated or deflated.

Irritant receptors are located throughout the epithelium of the upper and lower airway. After birth these receptors are stimulated by smoke, irritant gases or fluid and cause changes in frequency or depth of breathing. The afferent activity of these receptors is mediated by myelinated fibres of the nervus vagus. The response of the irritant receptors can be elicited after 35 weeks gestation in the human (Fleming

et al. 1978). In utero the irritant receptors are continuously exposed to lung fluid. The function (if any) of these receptors during fetal life is not known.

After birth mixed venous chemoreceptors and intrapulmonary chemoreceptors stimulate breathing activity (Sheldon & Green 1982). In utero pulmonary blood flow is only 20% of the right ventricular output. There is no data on the function of mixed venous and intrapulmonary chemoreceptors during fetal life. However, since vagotomy and denervation did not affect fetal breathing activity in utero, their function does not seem to be of importance. At birth there is a significant increase in pulmonary blood flow and the amount of CO_2 delivered to the lungs increases. If the CO_2 receptors are active they may play a powerful role in the stimulation of breathing activity at birth.

In summary, peripheral chemoreceptors (aortic and carotid), the vagus and its afferents seems to play a minor role in the regulation of spontaneous fetal breathing activity.

2.6 Neuromodulators and drugs

Many attempts have been made to understand the control mechanisms of breathing activity using pharmacological interventions.

Neurotransmitters e.g. norepinephrine, epinephrine, serotonin, act quickly over short synaptic distances. Neurohormones act at a distance from their place of release and cause more long lasting effects (e.g. adenosine, opioids, prostaglandins).

During HV ECoG plasma *norepinephrine and epinephrine* levels were higher than during LV ECoG (Reid et al. 1990). The effect of infusion of norepinephrine and epinephrine is to stimulate breathing activity (Bamford et al. 1986, Jansen et al. 1986, Moore et al. 1994). Isoproterenol stimulated breathing activity, but this might be related to the increase in brain metabolism (Murata et al. 1981, Jansen et al. 1986). Drugs which increased central catecholamines (DMI; desipramine, 6-OHDA; 6-hydroxydopamine) induced an increase of breathing activity associated with an increase in LV ECoG (Joseph & Walker 1990). At birth norepinephrine turnover increased significantly after 1-2 hours breathing air (Lagercrantz et al. 1992). A transient increase in catecholamines at the time of birth may thus contribute to the initiation of breathing activity.

Pilocarpine (cholinergic agonist) stimulated breathing activity in normal (Brown et al. 1981, Jansen et al. 1983, Hinman & Szeto 1988, Hanson et al. 1988) and chemodenervated fetal lambs in utero (Hanson et al. 1988). Pilocarpine appears to act above the level of the colliculi causing a change of ECoG activity to LV ECoG, and after infusing cholinergic antagonists ECoG activity switched into HV ECoG. Since pilocarpine also stimulated fetal breathing activity in brain stem transected fetuses, the medulla or pons appears to be involved (Hanson et al. 1988). Atropine blocked the pilocarpine induced breathing activity which

suggested that a cholinergic link may be involved in the regulation of fetal breathing activity (Hanson et al. 1988, Hinman & Szeto 1988).

Doxapram stimulated breathing activity in LV ECoG. The site of action must be the pons or the medulla since the response was unaffected by brain stem transection and chemodenervation (Jansen et al. 1983, Bamford et al. 1986).

Peripheral infusion of *5-hydroxytryptophan* (converted to serotonin) induced continuous breathing activity in fetuses >127 days of gestation. Furthermore, breathing activity was more regular and there was an increase in amplitude (Quilligan et al. 1981). Consistent with those results are the results of Fletcher et al. (1988) who infused 5-hydroxytryptophan together with antagonists of 5-hydroxytryptamine and blocked the stimulation of breathing activity.

During *thyrotrophin releasing hormone* (TRH) infusions breathing activity became continuous (Bennet et al. 1988), TRH positive fibres were located throughout the medulla-pons region of the rat brain (McCown et al. 1986). At birth, there was a rise of TSH which was dependent on the fall in environmental temperature experienced at birth (Sack et al. 1976). Since TRH was effective at very low doses it was speculated that TRH was involved in the switch from fetal to postnatal patterns of respiration at birth (Bennet et al. 1988).

Breathing activity became present continuously during infusions of *corticotrophin releasing factor* (CRF) and both frequency and amplitude increased (Bennet et al. 1990b). CRF antagonists inhibited fetal breathing activity during LV ECoG in normoxic fetal lambs, suggesting that CRF may have a tonic role in the generation of fetal breathing movements.

In the human, maternal consumption of *caffeinated and decaffeinated coffee* increased fetal breathing activity after 3 hours. Caffeine crossed the placenta rapidly. The 3-hour delay suggested that another mechanism played a role e.g. an increase in plasma glucose. Coffee raised slightly the maternal glucose level and the incidence and frequency of fetal breathing movements were significantly increased in response to glucose injections (Bocking et al. 1982). This increase in breathing activity was most probably due to increased concentrations of CO_2 which influenced the respiratory centre in the medulla of the fetal brain since glucose was oxidized aerobically within the fetus and metabolized to CO_2 and H_2O.

Theophylline, an adenosine antagonist, stimulated breathing movements during normoxia (Bissonnette et al. 1990, Bissonnette et al. 1991) and prevented the inhibition during hypoxemia by blocking the adenosine R_1 receptors (Hedner et al. 1982).

A biphasic breathing response (apnea followed by significantly deeper breathing movements) occurred when *morphine* was infused and this response was dose related. The apnea was correlated with the highest plasma concentrations and the onset of continuous breathing coincided with the lowest plasma concentration of morphine (Hasan et al. 1988). After brain stem section the period of apnea was significantly longer and continuous breathing activity did not occur which suggested that apnea was induced by inhibiting neurones directly, and continuous breathing

activity by disinhibition of more rostral neurones which inhibited the respiratory neurones (Hasan et al. 1990).

Fetal breathing activity was inhibited when the fetus was exposed to *pentobarbitone* and there was also a decrease in LV ECoG (Boddy et al. 1976).

PGE_2 is a well-known modulator of breathing. The fetal membranes and cotelydons are one of the major sources of circulating prostaglandins in the fetus (Olson et al. 1986). In newborn lambs breathing activity decreased during peripheral infusion of PGE_2 and increased during meclofenamate infusion (Guerra et al. 1988, Guerra et al. 1989). Several groups reported that administration of prostaglandin or prostaglandin synthetase inhibitors (indomethacin, meclofenamate) modulated fetal breathing movements (Kitterman et al. 1979, Kitterman et al. 1983, Wallen et al. 1986, Patrick et al. 1987, Hallak et al. 1992). Fetal breathing movements were even present in HV ECoG after infusion of indomethacin or meclofenamate (Kitterman et al. 1979, Wallen et al. 1986), which suggests that endogenous production of prostaglandins inhibits breathing activity in utero during HV ECoG and might be one of the mechanisms controlling breathing activity in utero. Prostaglandins act centrally in the lower pons or medulla to modulate fetal breathing movements (Jansen et al. 1984, Koos 1985). The mechanism by which they act might be on the respiratory nuclei, via the efferents or at the central chemoreceptors. In neonatal lambs the effects of PGE_2 were due to reduction of peripheral chemoreceptor discharge (Bennet & Hanson 1990a).

Prostaglandins do not appear to have effects on ECoG activity, rapid eye movements, blood pressure, heart rate or the excitability of the neural pathways of the cranial (digastric) or spinal (flexor) reflexes (Walker 1990).

Ventriculocisternal or i.v.infusion of *meclofenamate* resulted in breathing activity even during HV ECoG. These breathing movements became more regular during HV ECoG (as postnatally) than during LV ECoG. The incidence of breathing activity remained high during 60 hours of infusion (Wallen et al. 1988). This long-term effect of meclofenamate is opposite to the transient initial effect of indomethacin since infusion of indomethacin into the maternal femoral vein for 70 hours induced an increase in incidence and amplitude of breathing activity with a peak at 8 to 10 hours. However, but the effect was no longer present after 20 hours despite ongoing indomethacin infusion for a total period of 70 hours (Patrick et al. 1987). The human fetus showed a significant increase of breathing activity, but there was also no change in fetal movements (Hallak et al. 1992). A possible mechanism by which indomethacin increases the incidence of fetal respiratory activity might be by stimulating central chemoreceptors by producing cerebral acidosis (Hohimer et al. 1983, 1985). Altering glucose concentrations (Murai et al. 1984), changes in arterial blood gas tensions (Kitterman 1979, Murai et al. 1984, Koos 1985) and pH (Jansen et al. 1984, Hohimer et al. 1985, Wallen et al. 1986) or an increase in the incidence of LV ECoG activity (Kitterman et al. 1979) did not occur during infusion of prostaglandin

inhibitors and did not play a role in the increase of breathing activity even during HV ECoG. Meclofenamate is known to uncouple phosphorylation resulting in an increase in CO_2 production which could stimulate breathing activity (Whitehouse & Haslam 1962).

During the days before delivery breathing activity decreases significantly, and there is a significant increase of PGE_2. This suggested that prostaglandins play a major role in the control of breathing activity and at the time of birth. However, meclofenamate treated fetuses showed a similar significant decrease of breathing activity during the two days before delivery (Wallen et al. 1988). Therefore, the decrease of breathing activity before delivery is not dependent on the concurrent increase in PGE_2. Peripheral and central PGE_2 increased remarkably just before delivery and decreased during the first 24 hours rapidly after delivery. However, the establishment of continuous breathing at birth occurred when PGE_2 levels (peripheral or ventriculocisternal) were similar or higher than fetal levels (Jones et al. 1990, Lee et al. 1989). This suggests that the fall in PGE_2 level is not the primary mechanism involved in the establishment of continuous breathing at birth, but may play a role in the maintenance of continuous breathing.

In summary, a number of pharmacological agents stimulate breathing activity such as pilocarpine, opiates, doxapram, theophylline, caffeine, 5-hydroxytryptophan, TRH. Fetal breathing activity is depressed when the fetus is exposed to pentobarbitone, diazepam, adenosine (see hypoxemia) or PGE_2.

Many mechanims have been studied and proposed as responsible for the regulation of breathing activity in utero. It is difficult to draw firm conclusions since the actions and proposed mechanisms vary widely. It seems highly unlikely that so many different mechanisms are involved in the normal regulation of breathing activity in utero.

2.7 Birth

At birth, gas exchange must be achieved by the lungs. At birth many factors are involved in the stimulation of breathing activity such as extra afferent input, e.g. cooling, touch, pain, sound and light, which can contribute to the initiation of breathing or facilitate it by changing the sensitivity to CO_2. Furthermore, in the newborn there is an increase in systemic vascular resistance, a decrease in pulmonary vascular resistance and an increase in pulmonary blood flow (Iwamoto et al. 1987). After birth there is exclusion of the placenta from the circulation and levels of inhibitory substances produced by the placenta fall which may allow continuous breathing activity (Adamson et al. 1987, Blanco et al. 1987b, Adamson et al. 1991). Umbilical cord occlusion and increased pulmonary blood flow may result in decreased level of fetal prostagladins (Adamson et al. 1991, Ferreira & Vane 1967). This led to the suggestion that a decrease of placental factors secreted into the plasma is involved in the initiation of continuous breathing at birth.

2.7.1 Extra afferent input

In utero there are only small changes in temperature and other conditions in the environment are quite stable. The change in environment at birth exposes the fetus to extra afferent input produced by cooling, touch, pain, light and sound which may play a role in the initiation of continuous breathing activity. Peripheral nerve electrical stimulation resulted in continuous breathing activity in fetal sheep (Condorelli & Scarpelli 1975, Scarpelli et al. 1977) but these results could not be confirmed by others (Blanco et al. 1983b). Stimulation of the fetus by sound resulted in a significant change in ECoG state from HV ECoG to LV ECoG associated with breathing activity, rapid eye movements and nuchal muscle activity suggesting arousal (Fletcher et al. 1989, Parkes et al. 1991). Extra afferent input might change thresholds and the arousability of the newborn, resulting in continuous breathing.

Newborn lambs respond to cooling by increasing pulmonary ventilation (Andrews et al. 1991) and oxygen consumption (Alexander & Williams 1968, Dawes et al. 1968, Sidi et al. 1983, Andrews et al. 1991). The increase in ventilation was caused by an increase in breath amplitude (young lambs) or in older lambs an increased amplitude in combination with a higher frequency (Andrews et al. 1991). It has been concluded that metabolic rate is a major stimulus in modulating breathing activity (Andrews et al. 1991). When the newborn lamb was exposed to cooling, there was also an increase in heat production, in blood pressure and heart rate (Sidi et al. 1983) and in plasma norepinephrine levels (van Bel et al. 1993). These responses are mature before birth in the ovine fetus (Gluckman et al. 1983, Gunn et al. 1985, Kuwamura et al. 1986). Although thermogenic responses are not needed for fetal survival, they are essential for neonatal adaptation since the newborn is exposed to an abrupt fall in environmental temperature at birth.

Reduction of fetal skin temperature induced regular rhythmic breathing activity in utero even during HV ECoG (Harned & Ferreiro 1973, Gluckman et al. 1983). Breathing started in externally cooled fetuses before there had been a fall of fetal central temperature or amniotic temperature. It was suggested that breathing activity had been induced as a consequence of increased afferent input from cutaneous thermoreceptors which could have resulted in arousal. Interestingly, the effects were inhibited during hypoxemia which again suggested that central control of fetal breathing activity overrides peripheral control.

Slow external cooling produced by a coil around the fetus had no effect on breathing activity. Slow internal and rapid internal cooling had no effect on the periodicity of fetal breathing movements and breathing movements remaining associated with LV ECoG (Gluckman et al. 1983). The results of these experiments were limited by the fact that the internal coil was in contact with the pharynx, larynx, oesophagus and ended in a loop inside the stomach. This might have influenced breathing activity during cooling periods by direct stimulation of sensitive upper airway areas.

The mechanism by which cooling changes the pattern of breathing in utero is not known. It could be via direct changes in arousability as described but also one can imagine that the sensitivity to CO_2 might have changed. It was reported by Moss et al. (1983) that in acute experiments the normally high CO_2 threshold of the fetus (7.4 ± 0.3 kPa) was lowered by peripheral cooling and a decrease in core temperature. Fetal placement in a cool bath produced immediate wakefulness and onset of breathing which was attributed to a cutaneous receptor reflex. When the newborn was exposed to peripheral and central cooling there was an increase in muscle movements, shivering, and an increase in oxygen consumption which resulted in an increase in CO_2 production. More regular breathing movements occurred in association with central core cooling and peripheral cooling (Moss et al. 1983). Moreover, it is reported that at birth, at the time of initiation of breathing, cold could override the negative effects of hypocapnia or moderate hypoxia (Blanco et al. 1987b).

In summary, peripheral afferent input may change thresholds involved in the initiation and maintenance of continuous breathing at birth. Thus a decreased temperature may play a role in the initiation of breathing activity directly or may change the sensitivity to CO_2 and override the inhibitory effects of HV ECoG.

2.7.2 Umbilical cord occlusion

In utero gas exchange is regulated by the placenta. The placenta is known to produce many hormones which could modulate fetal breathing activity, e.g PGE_2, endorphins, progesterone, adenosine etc. After cord occlusion there is a decrease in concentrations of plasma levels which could allow the establishment of continuous breathing at birth.

The existence of a placental factor modulating breathing activity in utero was proposed by Adamson et al. (1987) and Blanco et al. (1987b). In their experiments breathing activity became continuously present after cord occlusion and breathing activity ceased on the release of the cord in mechanically ventilated fetal lambs. This suggested that modulators were restored to the fetal circulation when the cord was released and that these modulators inhibited breathing activity in utero. However, during these experiments $PaCO_2$ increased and pH decreased. Therefore, the presence of these confounding changes in these experiments make them inconclusive.

However, other investigators reported that during cord occlusions, when fetal PaO_2 remained in the normoxic range (2-3 kPa) and $PaCO_2$ rose to 9-12 kPa, there was *no* continuous breathing activity (Faber et al. 1985, Baier et al. 1990, Alvarez et al. 1992). Recently, Adamson et al. attempted (1991) to keep $PaCO_2$ unchanged during cord occlusion by using high frequency oscillation (HFO). Under these conditions cord occlusion produced an increase in the incidence, frequency and amplitude of fetal breathing movements during a 30 min cord occlusion compared to a 20 min control period. The increase was larger in the last 10 min than

during the first 10 min of the 30 min cord occlusion. This suggests that other factors are likely to be responsible for the initiation of breathing at birth since continuous breathing activity is normally initiated within 5 min of cord occlusion (Adamson 1993). Since these experiments require expansion of the lung with air new variables were added to the experiments, e.g. pulmonary circulation increased and the airways were in contact with changes in CO_2 (Marsland et al. 1975, Haddad & Mellins 1977, Banzett et al. 1978, Sheldon & Green 1982).

Alvaro et al. (1993) reported that they could obtain inhibition of breathing activity by infusion of a placental extract in fetal sheep. These experiments did have a lack of specificity since they did not single out a possible substance which might have been involved. The placenta could certainly contain substances which inhibit breathing activity but Alvaro et al. did not test whether extracts of other organs had an effect on fetal breathing activity, for example an extract of brain, liver etc. Furthermore, fetal PaO_2 in these experiments was in the range of 27-45 kPa a level which would never normally be reached in prenatal or postnatal life. The physiological activity of substances in the extracts at a significantly lower pH and an unphysiologically high PaO_2 might therefore be different from the physiological activity of substances which were released normally by the placenta in the normoxic-normocapnic fetus.

In summary, it is clear that at the moment of birth or shortly thereafter there are changes in oxygen consumption and CO_2 production. These changes could certainly have an important role in the establishment and maintenance of breathing at birth. Cord occlusion experiments may have introduced confounding variables since blood gases were not well controlled and the lungs were exposed to mechanical ventilation, CPAP and the airways were exposed to changes or increased levels of CO_2. Use of techniques which control fetal blood gases without pulmonary ventilation could offer the solution to the question of whether exclusion of the umbilical circulation *per se*, or a rise in $PaCO_2$ during this transition, play a role in the initiation of breathing at birth.

Chapter 3

Materials & Methods

3.1 Animals

Experiments were performed on unanesthetized chronically instrumented fetal sheep in utero. The chronic fetal sheep preparation has been used for the past 20 years to study fetal and neonatal physiology. The reason for this is that many techniques are well established and documented: the fetus is large enough to be instrumented, the ewe tolerates abdominal surgery well, will stand and eat within 30 min of recovery from anesthesia, adapts well to living in metabolic cages and is not prone to abortion following surgery.

3.2 Surgical procedure

Pregnant sheep were operated at 128-132 days of gestational age after 48 hours fasting, using sterile technique and under general anesthesia. The anesthesia was induced with Sodium Thiopentone (Nesdonal) 1g/70kg i.v. The ewe was intubated and maintained during the operation with 0.8% Halothane in N_2O and O_2 (50%/50%). Catheters (polyvinyl, 1 mm ID) were placed in the ewes carotid artery and jugular vein for blood sampling or infusing antibiotics. A midline laparotomy was performed and the uterus was partly exteriorized to allow access to the fetal head. The uterus was opened and the fetus was exteriorized down to the umbilical cord. The volume required for total occlusion of an inflatable cuff occluder (VO-4, 2 cm ID, Rhodes Medical Instruments, Woodland Hills, U.S.A.) was checked by inflating the occluder with saline until the lumen was closed. The occluder was placed around the umbilical cord and anchored to the abdominal skin. A fetal thoracotomy was done at the level of the 10th intercostal space. Four wire electrodes (AS 632 Cooner Wire, Chatsworth, U.S.A.) were sewn in the diaphragm muscle to record EMG activity and a thermistor (PT 100 sensors, Murata, Nijkerk Electronics, The Netherlands) was left in the pleural cavity to measure fetal central temperature. The thorax was closed. Polyvinyl catheters (0.8 mm ID) were placed in the right brachial artery to measure blood pressure, heart rate and for fetal blood sampling. A catheter (polyvinyl, 0.8 mm ID) was placed in the right brachial vein to infuse antibiotics and fluids. Two wire electrodes for recording ECoG activity were implanted bilaterally on the fetal parietal dura through holes (1 mm) drilled in the skull. A common electrode was sewn in adjacent tissue. Two wire electrodes were sewn into the periorbital muscles (vertical) to record eye movements. An incision was made at the level of the nuchal extensor muscles and 2 wire electrodes were sewn into these muscles to record EMG activity. A catheter (polyvinyl, 0.8 mm ID) was placed in the fetal trachea to measure intratracheal pressure.

The fetal lambs were heparinized (bolus 100 U/kg for the approximated weight) after isolating the carotid artery and jugular vein just before placing catheters in these vessels. A polyvinyl catheter

(0.8 mm ID) was placed in the right carotid artery (directed towards the head) to measure blood pressure, heart rate and for fetal blood sampling. For the ECMO system two catheters (Biomedicus, Eden Prairie, U.S.A.) were placed at midcervical level; one for drainage to the ECMO system (10-14 French, multiple holes) was advanced 10 cm into the right external jugular vein to the right atrium level and one for return of oxygenated blood to the fetus (8-10 French, end hole) was advanced 3 cm into the right carotid artery towards the aortic arch. These catheters were connected to 60 cm silastic tubes (0.65 cm ID, Medical Grade Tubing, Dow Corning, Midland, U.S.A.) capped at the end and filled with heparinized saline (50 U/ml). These tubes permitted later connection to the ECMO circuit.

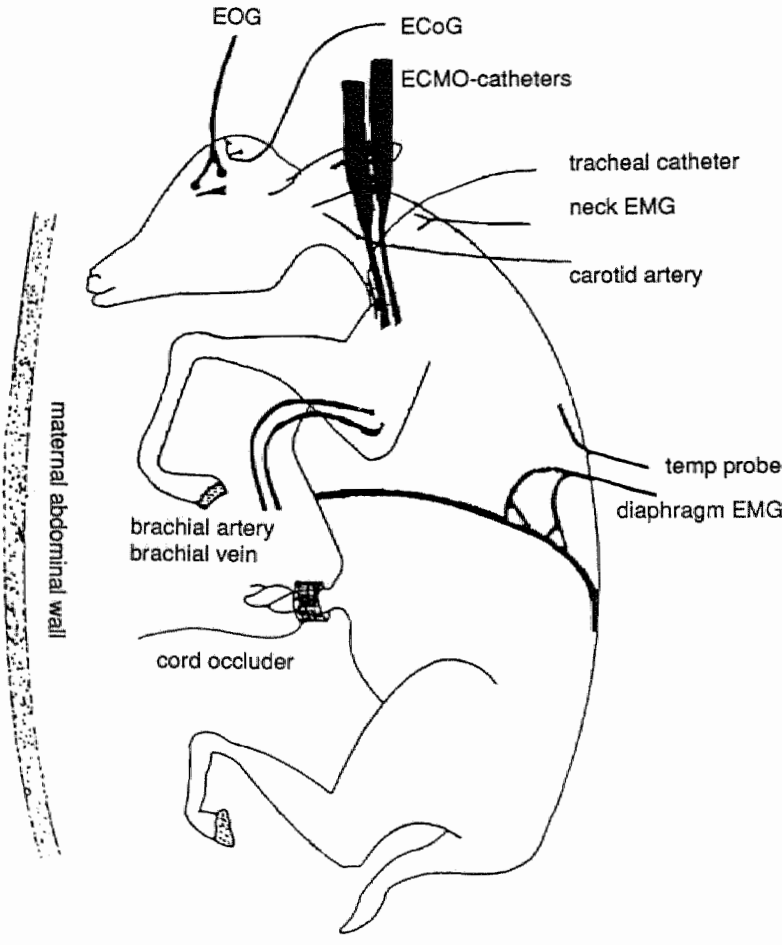

Figure 3.1 Illustration of the final stage of the fetal chronic preparation as described.

After the instrumentation was completed, the fetus was returned to the amniotic cavity. Amniotic fluid loss was replaced by warm saline. The uterus was sewn in 2 layers. All catheters, tubing and electrodes cables were exteriorized through a small incision in the flank of the ewe and stored in a plastic/cotton bag sewn adjacent to the exteriorized site. The abdomen was sewn in layers. The procedure lasted for about 3-3.5 hours. Figure 3.1 shows an instrumented fetus.

Postoperatively, the ewe was placed in a cage in the experimental room. The ECMO catheters were flushed twice daily with heparinized saline (5 ml, 50 U/ml) and both catheters were connected to a continuous infusion of heparinized saline (1 ml/hr, 50 U/ml). Antibiotics were given daily to the ewe (ampicillin 1 g/day) and the fetus (ampicillin 150 mg/kg/day and gentamicin 5 mg/kg/day) until the end of experimentation.

The success rate of this preparation was reasonable (50%). During surgery placing of the ECMO catheters was sometimes difficult due to their size and stiffness after resterilization. Another reason for failure of the preparation was hemorrhage, due to heparinization. Furthermore, despite the fact that the ewes were not prone to abortion after surgery, some ewes were in labour at the time of experimentation due to the timing of the surgery (third trimester).

3.3 Extracorporeal membrane oxygenation system

The ECMO system is essentially a modified heart-lung machine. The system consisted of 0.65 cm ID silastic tubing (Medical Grade Tubing, Dow Corning, Midland, U.S.A.), a venous reservoir (bladder, 50 ml silastic reservoir, Scimed Life systems, Minnesota, U.S.A.), a raceway (silastic tubing exposed to the roller heads of the pump, 0.95 cm ID, Medical Grade Tubing, Dow Corning, Midland, U.S.A.), a peristaltic pump (Travenol), a membrane lung (Scimed 0.8 m^2, Scimed life systems, Minnesota, U.S.A.), connectors (Baxter, Uden, the Netherlands), stopcocks (Baxter, Uden, the Netherlands), and pigtails (stopcock with 10 cm extension, Vigo-Spectromed, Heisingborg, Sweden). The circuits were made according figure 3.2 and later sterilized (the membrane lung was delivered sterile). Three venous ports before the roller pump were placed in the circuit. One port was for temperature measurement of the blood, one port was for venous sampling and one port was for continuous infusion of heparin. One venous port was placed in the circuit before the membrane for pre-membrane pressure monitoring. One arterial port was placed after the membrane for post-membrane monitoring.

3.3.1 Priming of the Circuit

There are 3 phases of priming procedure: gas prime, crystalloid prime and blood prime. For the gas prime, CO_2 is used since CO_2 replaces N_2 and CO_2 is more soluble in liquid. The crystalloid prime is used to fill the

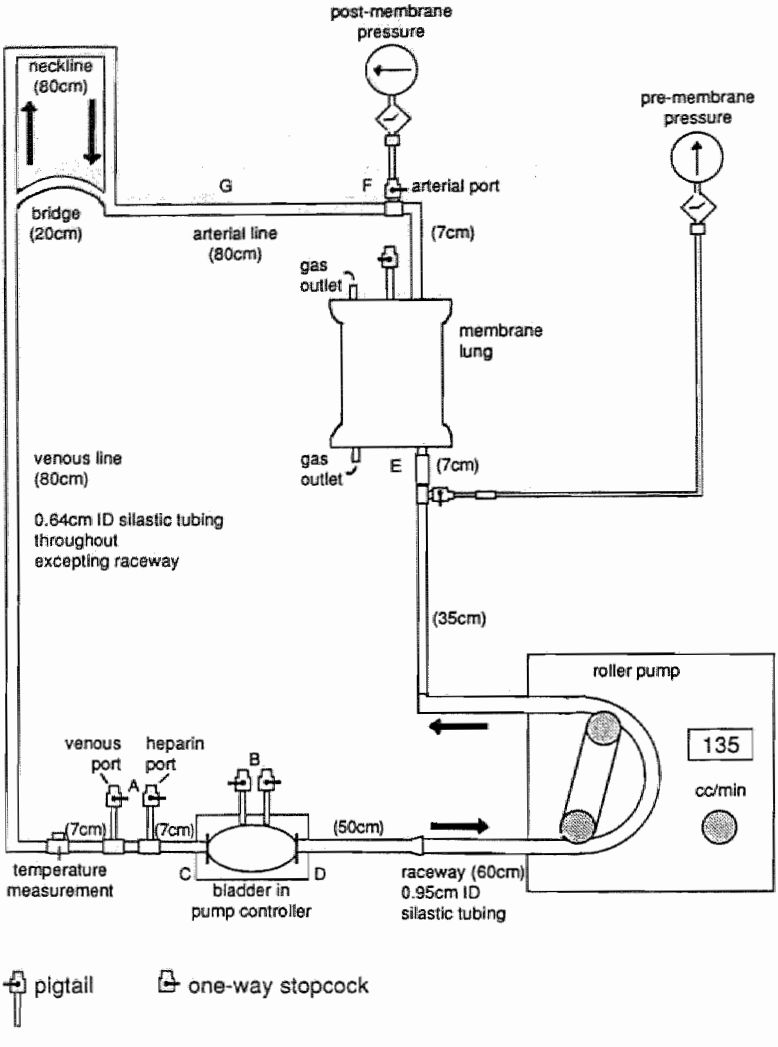

Figure 3.2 Diagram of the ECMO circuit made for the experiments. The capital letters correspond to specific tasks in the priming procedure.

circuit with a balanced electrolyte solution in a bubble free way and to coat the circuit.

The gas prime

CO_2 was introduced in the blood path of the circuit at a flow of 2 l/min CO_2 via a filter connected to one of the stopcocks (B) of the bladder and at the same time the arterial port (F) was open. After 3 min a clamp was placed on the arterial line (G) between the arterial port and the

bridge. Therefore, CO_2 passed through the membrane lung. Three minutes later the CO_2 flow was stopped and the stopcock (B) of the bladder and the arterial port (F) were closed simultaneously. The clamp was removed. Wall suction was connected to the gas inlet and the gas outlet of the membrane lung. Applied suction did not exceed 30 mmHg to prevent damaging the silicone membrane. At this point the bladder collapsed.

The crystalloid prime

Haemaccel (Behring, Pharma Hoechst, the Netherlands) was used to fill the system, connected via an infusion set to a stopcock (B) of the bladder. One clamp was placed on the inlet (C) of the bladder and one clamp on the outlet (D) of the bladder. The stopcock (B) was opened and the bladder was filled with Haemaccel. The negative pressure within the system generated by wall suctioning drove this part of the priming. The circuit was filled in two parts, retrograde up to the arterial port (F) and antegrade through the membrane till the arterial port (F).

Retrograde: the clamp on the inlet (C) of the bladder was opened slowly to avoid bubble formation. The venous line, neckline, bridge and arterial line were filled until the arterial port (F).

Antegrade: the outlet clamp was removed, the raceway was filled and the membrane was filled slowly. After the membrane was filled completely with Haemaccel, the line was filled till the arterial port (F). Suction was disconnected. Gentle tapping on the membrane mobilized any trapped air in the membrane. By briefly opening each stopcock of the pigtails each pigtail was filled with Haemaccel. All clamps were removed from the circuit, the raceway was placed in the pump, the pump was started, the prime was circulated.

The blood prime

One unit of fresh anticoagulant citrate dextrose (ACD) adult sheep blood (non-pregnant) was prepared. The blood was prepared by adding 25 ml of tromethamine (THAM), 20 ml of $NaHCO_3$ (4.2%), 50 ml of 20% albumin, 100 units of heparin to each unit. After rocking the bag thoroughly but gently, 300 mg Calcium gluconate was added to each bag. Calcium gluconate was always added after heparin since it reverses anticoagulant activity in the blood.

The blood bag was connected by a new infusion set to one of the stopcocks (B) of the bladder. An empty bag was connected via a connecting system to the heparin port (A). A clamp was placed on the inlet (C) of the bladder, the stopcock (B) to the bloodbag was closed, and the stopcock (A) to the empty bag open. The pump was started carefully, until collapsing of the bladder, then the pump was turned off. A clamp was placed on the outlet (D) of the bladder, the stopcock (B) to the bladder was opened and the bladder was filled with blood. When the bladder was full with blood the clamp on the outlet (D) of the bladder was removed, and the pump turned on 50 ml/min. This part of the priming is pump-driven. The blood was circulating slowly into the circuit,

and a Haemaccel-blood interface was maintained. The flow of blood was traced along the arterial line (G) to the bridge and the bridge was filled and clamped. The blood was traced along the necklines and back down to the heparin port (A). When the Haemaccel had been totally replaced by blood and extra blood was purged at the heparin port (A), the pump was turned off, the stopcock of the heparin port (A) and the stopcock (B) of the bladder were closed. All clamps were removed, the pump was turned to 200 ml/min. The system was checked for air and all bubbles were removed.

The pre- and post-membrane pressures were connected and monitored. Monitoring was useful for the following reasons. Transmembrane pressure was usually between 50-150 mmHg. An acute rise in pre-membrane pressure, not associated with an increase in pump flow, was predictive of clots forming in the membrane. Post-membrane pressure was indicative of obstructions on the arterial side of the circuit, for example kinking of the arterial side or clots in the arterial catheter.

Additional volume was added to the circuit to bring the postmembrane pressure to 100 mmHg at a pump flow of 200 ml/min. These adjustments prevented nett loss of volume from the fetus into the circuit at the time of initiating bypass.

Finally, the gas flow was connected to the membrane lung. In order to maintain fetal normocapnia and normoxia after connection of the fetus to the ECMO circuit the membrane lung was supplied with 0.4 l/min O_2, 1.5 l/min N_2 and 0.1 l/min CO_2 at a total flow rate of 2 l/min. The circuit was enclosed in a thermostatically controlled box which maintained the blood at 39.5 ^{0}C (equivalent to fetal central temperature).

The neckline was clamped with 2 clamps; one on the venous side and one on the arterial side. Both the ECMO catheters were clamped, and cleaned with 75% ethyl alcohol. The silastic tubing of the venous ECMO catheter was connected to the venous side of the neckline with a connector (Baxter, Uden, the Netherlands), the silastic tubing of the arterial ECMO catheter was connected to the arterial side of the neckline with a connector. The clamps of the ECMO catheters were removed. The pump flow was set to 50 ml/min, the clamp on the arterial neckline was removed, the main bridge was clamped and the clamp on the venous line was removed. The blood was drained from the right atrium via the jugular vein down the venous line to the bladder, passed the roller pump, the membrane lung and returned via the arterial line through the common carotid catheter to the aorta (figure 3.3). Slowly the pump flow was increased to approximately 150-200 ml/min within 15 min. At this flow the ECMO system took over ca. 15-20% of the fetal combined ventricular output (Cohn et al. 1974).

Heparinized (150 U/ml) saline was infused continuously via the heparin port at a flow of 2 ml/hr, to maintain activated coagulation time (ACT) at 250-350 seconds.

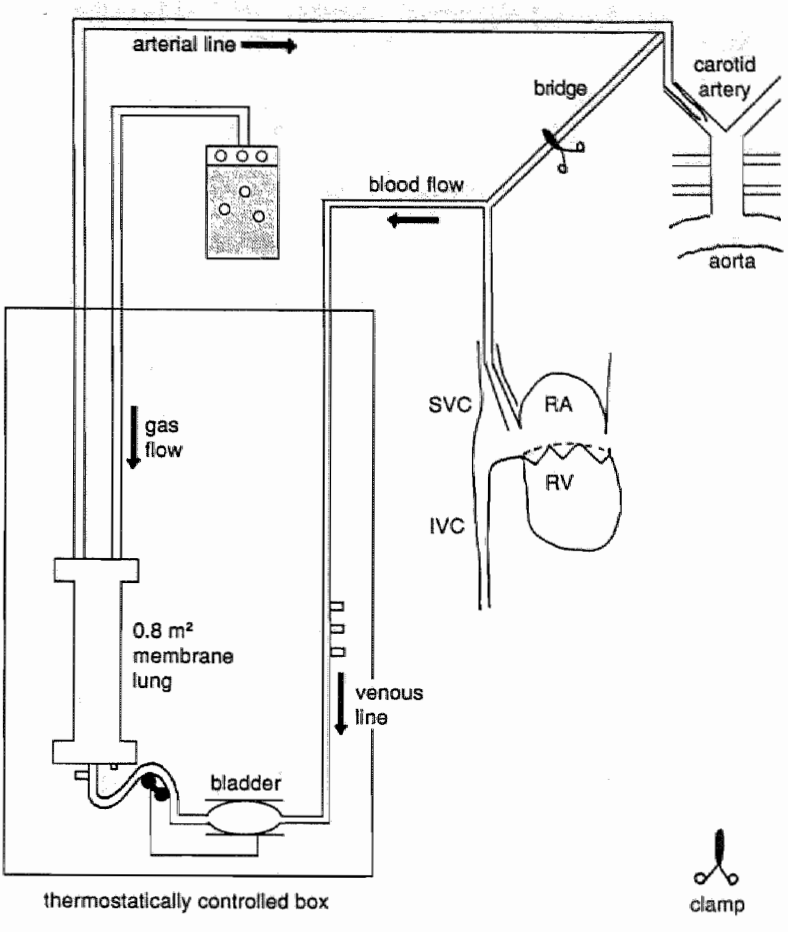

Figure 3.3 Diagram of the ECMO-system used for the experiments. Venous blood was drained from the right atrium and followed the venous line to the bladder, the roller pump, the membrane lung and then returned via the arterial line into the carotid artery of the fetus.

3.4 Data collection

Recordings were started within 48 hours after the operation. This was 24 hours before connection to the ECMO system. We used an 8 channel pen recorder (Hewlett-Packard; 7758 A recorder) at a paper speed of 1 cm/minute and the signals were also stored on tape (Hewlett-Packard 3968 A) for further analysis.

The following parameters were recorded continuously

1 Electrocortical activity: obtained from bipolar electrodes amplification for scale 100uV/2cm (filters: 4-40 Hz).
2 Integrated electromyographic activity of the posterior neck muscles (filters: 100-1000 Hz, raw signal integrated with a leaky integrator, integrator time constant 1 sec).
3 Electromyographic activity of the diaphragm muscle (filters: 100-1000 Hz, raw signal integrated with a leaky integrator, integrator time constant 0.3 sec).
4 Electromyographic activity of the periorbital muscles (filters 0.5-40 Hz).
5 Tracheal pressure; scale 0-40 mmHg, where negative pressure deflections indicated breathing movements.
6 Blood pressure; measured by an artery catheter, scale 0-100 mmHg.
7 Heart rate; obtained from the pulse pressure signal of the blood pressure, scale 0-400 bpm.
8 Central temperature; measured from a thermistor in the pleural cavity, scale 20-50 ^{0}C, and digitalized.

Blood samples (0.4 ml) were drawn from an arterial catheter to measure blood gas tensions and pH by using standard electrodes (ABL-3, Radiometer, Copenhagen) with values corrected to fetal temperature (39.5 ^{0}C). Blood samples were drawn (0.4 ml) from the venous port to measure ACT (Hemochron blood coagulation tubes P214, Hemochron 400).

3.5 Experimental protocol and analysis of results

Both integrated EMG activity of the diaphragm and the negative tracheal pressure deflections represented respiratory output. Fetal breathing was defined when activity of the diaphragm and/or repeated negative deflections of tracheal pressure were present for at least one minute (>6 breaths/min). Fetal ECoG was analyzed visually into LV ECoG and HV ECoG activity.

These recordings allowed to us obtain baseline data on incidence of fetal breathing activity, its relationship with LV ECoG activity and the distribution of fetal states before connecting the fetus to the ECMO system. A typical recording of a fetus 48 hours after surgery is shown in figure 3.4. On the third day post-surgery, when the fetal blood gases were within the normal range and when a baseline recording of >10 hours had shown that fetal breathing movements were present at a normal incidence, the fetuses were connected to the ECMO system, maintaining fetal temperature, blood gases and pH. Baseline recordings on ECMO were started at least one hour after connection to the ECMO system.

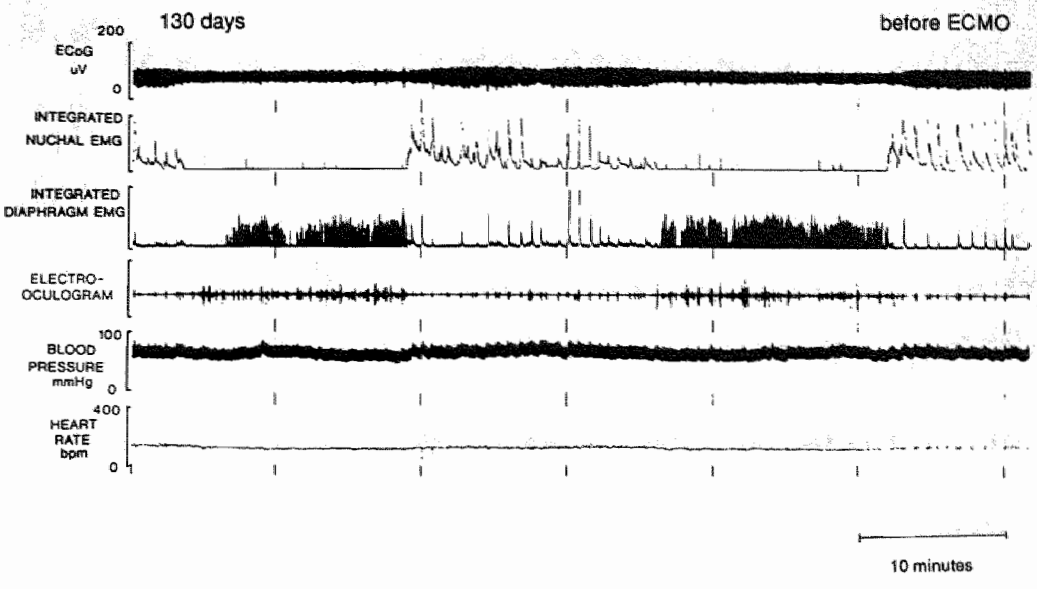

Figure 3.4 Typical recording of approximately one hour of a fetus of 130 days GA, 48 hours after the operation. Tracings are from top to bottom: ECoG activity, integrated nuchal EMG, integrated diaphragmatic EMG, electro-oculogram, blood pressure and heart rate. ECoG activity is differentiated into LV ECoG and HV ECoG activity. Fetal breathing movements and rapid eye movements are associated with LV ECoG activity, and nuchal EMG activity is associated with HV ECoG.

3.6 Statistics

All data are given as means ± SEM. In statistical analysis of data n was taken as the number of fetuses, not the number of experiments. Repeated trials on individual fetuses were averaged before statistical analysis. Non-parametric tests, i.e. Friedman and Wilcoxon signed rank test, were used for statistical comparison between the periods of the protocol.

Chapter 4

The effect of mild hypocapnia on breathing and behavior in unanesthetized normoxic fetal lambs

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Abstract

We hypothesized that the level of $PaCO_2$ affects the incidence of fetal breathing movements and ECoG states in chronically instrumented fetal sheep.

6 Fetuses at 128-132 days gestation were instrumented for recording fetal behavior and for later connection to an ECMO system to change fetal blood gases. Before ECMO fetal arterial pH and blood gases were: pH 7.40 ± 0.01, $PaCO_2$ 5.7 ± 0.20 kPa and PaO_2 2.56 ± 0.23 kPa; during ECMO in normocapnia they were: pH 7.37 ± 0.01, $PaCO_2$ 6.14 ± 0.09 kPa and PaO_2 3.68 ± 0.40 kPa: during ECMO in mild hypocapnia they were: pH 7.47 ± 0.01, $PaCO_2$ 4.71 ± 0.23 kPa and PaO_2 3.55 ± 0.23 kPa.

The overall incidence of breathing movements, the incidence of breathing movements during LV ECoG and the mean duration of periods of breathing decreased significantly during hypocapnia. Fetal ECoG activity showed normal cycling during the periods of mild hypocapnia and the mean duration of LV ECoG periods did not change. During mild hypocapnia, rapid eye movements (n=3) remained associated with LV ECoG and nuchal muscle activity with HV ECoG. These results suggest that the presence of breathing movements in fetal life is not only dependent on the behavioral state but also on the level of fetal $PaCO_2$.

Introduction

Fetal breathing movements are present from early in gestation (Cooke & Berger 1990, Dawes et al. 1972, Szeto et al. 1992) and are associated with phasic electro-ocular and tonic nuchal muscle activity (Clewlow et al. 1983). The function and regulation of these fetal breathing movements are unknown since gas exchange is regulated by the placenta. It has been suggested that fetal breathing movements are necessary for normal lung development but the mechanism is unknown (Fewell et al. 1981a, Liggins et al. 1981b). The first clear evidence of some regulation of breathing activity in utero occurs at the time of maturation of electrocortical activity into LV ECoG activity and high voltage electrocortical HV ECoG activity (Dawes et al. 1972, Ioffe et al. 1987). Breathing movements are present for only 30-40% of the total time and are associated with rapid eye movements and LV ECoG (Dawes et al. 1972). During LV ECoG breathing movements are present for approximately 65% of the time (Ioffe et al. 1980) while during HV ECoG breathing movements are inhibited (Dawes et al. 1983). Thus breathing movements have been suggested to be part of the expression of fetal behavior (Dawes et al. 1983, Jansen et al. 1981).

It is well known that the level of $PaCO_2$ regulates breathing activity postnatally (Berger et al. 1977). It has also been demonstrated that oscillations in $PaCO_2$ level are important in the regulation of breathing

after birth (see Kolobow et al. 1977, Phillipson et al. 1981). However, the role of the $PaCO_2$ *level* in determining the incidence of fetal breathing activity in late gestation, when breathing activity became episodic, is not firmly established. It is well known that hypercapnia stimulates breathing from early in gestation (Boddy et al. 1974, Connors et al. 1988, Ioffe et al. 1987). It has been shown that maternal hypocapnia produced by hyperventilation results in a decrease of breathing activity of the human and sheep fetus during the last weeks of gestation (Boddy et al. 1974, Connors et al. 1988, Marsál et al. 1979). However, hypocapnia is known to cause a decrease in uterine (Oakes et al. 1976, Walker et al. 1976) and in umbilical (Oakes et al. 1976) blood flow. Thus the fetus might have been hypoxemic (Oakes et al. 1976) and this is known to inhibit fetal breathing movements (Boddy et al. 1974, Koos et al. 1987a). In order to avoid these confounding variables we used an ECMO system in chronically instrumented fetal lambs. This allowed us to decrease fetal $PaCO_2$ while maintaining fetal oxygenation independently of the ewe. This allowed us to address the hypothesis that the $PaCO_2$ level in utero determines the incidence of fetal breathing movements.

Materials and methods

Experiments were performed on unanesthetized chronically instrumented fetal sheep in utero (see Chapter 3).

Recordings were obtained before ECMO, and on ECMO during normocapnia ($PaCO_2$ between 5.5-6.5 kPa) and mild hypocapnia periods. Mild hypocapnia was obtained by decreasing the CO_2 flow to the membrane lung. Mild hypocapnia was defined as a fetal $PaCO_2$ 1 to 2 kPa less than baseline.

Fetal blood gases and pH, the incidence per hour and the length of periods of LV ECoG, the incidence per hour and length of periods of fetal breathing movements, and the incidence of fetal breathing movements during LV ECoG were measured during a) periods before ECMO, and on ECMO b) during normocapnia periods and c) mild hypocapnia periods. Recordings also continued for a period after return to fetal normocapnia. Mean arterial blood pressure and heart rate were analyzed every 10 min. Amniotic pressure was not subtracted from the blood pressure.

The Friedman and Wilcoxon signed rank test were used for statistical comparison of variables between the periods of the protocol.

Results

Experiments were performed on 6 fetal lambs, at gestational age of 131-135 days.

Baseline recordings before connecting the fetus to the ECMO system.

Baseline recordings were obtained 55 to 70 hours after the operation, the duration of these recordings being 11 hrs to 21 hrs. Fetal pH and blood gases were: pH 7.40 ± 0.01, $PaCO_2$ 5.70 ± 0.20 kPa, and PaO_2 2.56 ± 0.23 kPa. Mean arterial blood pressure was 67.2 ± 4.3 mmHg, and heart rate was 157 ± 9.1 bpm.

Nuchal EMG activity was associated with HV ECoG. Rapid eye movements (3 fetuses) were associated with LV ECoG activity and fetal breathing movements. LV ECoG activity occurred 49.3 ± 2.4% of the time and the duration of the LV ECoG periods was 14.6 ± 0.6 min. Fetal breathing activity was present for 36.0 ± 2.8% of the time, the length of the fetal breathing periods being 10.2 ± 1.1 min. The incidence of breathing activity during LV ECoG was 72.8 ± 3.6%.

Normocapnia periods on ECMO

On the third day post-surgery the fetuses were connected to the ECMO system. These periods of normocapnia were studied to control for possible effects of ECMO on normal fetal behaviour. Fetal pH and blood gases during these baseline recordings on the ECMO system were: pH 7.37 ± 0.01, $PaCO_2$ 6.14 ± 0.09 kPa, and PaO_2 3.68 ± 0.40 kPa. Mean arterial blood pressure was 72.8 ± 5.9 mmHg, and heart rate was 168.4 ± 10.7 bpm.

Nuchal EMG activity was associated with HV ECoG activity. Rapid eye movements (n=3) were associated with LV ECoG activity and fetal breathing movements. The incidence of LV ECoG was 46.6 ± 2.5% of the time and the length of the LV ECoG periods was 15.5 ± 1.1 min (see table 4.1). Breathing activity was present for 34.6 ± 3.0% of the time, the length of fetal breathing periods being 9.3 ± 1.2 min. The incidence of breathing activity during LV ECoG was 75.5 ± 2.6%.

Table 4.1 **The effect of mild hypocapnia on fetal blood gases and pH, fetal behavior and breathing activity in unanesthetized fetal lambs.**

Physiologic variables	*Normocapnia on ECMO*	*Mild hypocapnia on ECMO*
pH	7.37 ± 0.01	7.47 ± 0.01[1]
$PaCO_2$ (kPa)	6.14 ± 0.09	4.71 ± 0.23[1]
PaO_2 (kPa)	3.68 ± 0.40	3.55 ± 0.23
LV ECoG (%)	46.6 ± 2.5	45.5 ± 1.4
LV ECoG, LP (min)	15.5 ± 1.1	13.6 ± 1.0
FBM (%)	34.6 ± 3.0	14.3 ± 3.9[1]
FBM periods, LP (min)	9.3 ± 1.2	4.9 ± 1.2[1]
FBM/LV ECoG (%)	75.5 ± 2.6	32.2 ± 9.5[1]

Means ± SEM of fetal blood gases and pH, incidence and length of periods of LV ECoG and fetal breathing movements, the incidence of fetal breathing movements during LV ECoG (FBM/LV ECoG). (LP; length of periods, FBM; fetal breathing movements, %; percentage of the time present, [1] $p<0.05$, mild hypocapnia periods on ECMO compared to normocapnia on ECMO).

Mild hypocapnia periods on ECMO

A total of 9 experiments were performed in 6 fetuses. Figure 4.1 shows 60 min from one experiment. There was more than one experiment performed on 3 animals: the time between experiments was 7 hours to 29 hours. The duration of mild hypocapnia periods ranged from 2 hours to 10 hours. Fetal pH and blood gases were: pH 7.47 ± 0.01 ($p< 0.05$ compared to normocapnia period on ECMO), $PaCO_2$ 4.71 ± 0.23 kPa ($p<0.05$) and PaO_2 3.55 ± 0.23 kPa. Mean arterial blood pressure was 69.8 ± 3.9 mmHg, and heart rate was 164.8 ± 14.7 bpm.

Nuchal EMG activity was present and associated with HV ECoG. Rapid eye movements (n=3) were present during LV ECoG activity. The incidence of LV ECoG was 45.5 ± 1.4% of the time, the duration of the LV ECoG periods was 13.6 ± 1.0 min (see table 4.1.). The incidence of breathing activity was 14.3 ± 3.9% ($p<0.05$) and the length of the fetal breathing periods was 4.9 ± 1.2 min ($p<0.05$). The incidence of breathing activity during LV ECoG was 32.2 ± 9.5% ($p<0.05$).

When fetal $PaCO_2$ was returned to control breathing activity was present (2 experiments) or reappeared within 4 min (7 experiments). These breathing movements were only present during LV ECoG, and were stopped with the occurrence of HV ECoG activity. Figure 4.2 shows

an example of the reappearance of fetal breathing activity after increasing fetal $PaCO_2$. During the first hour immediately after mild hypocapnia, the incidence of breathing activity was 54.6 ± 1.2% ($p<0.05$, compared to normocapnia on ECMO) and the incidence of LV ECoG was 62.4 ± 1.9% ($p<0.05$). During the first hour after mild hypocapnia fetal pH and blood gases were: pH 7.37 ± 0.01, $PaCO_2$ 6.00 ± 0.15 kPa, and PaO_2 4.08 ± 0.37 kPa.

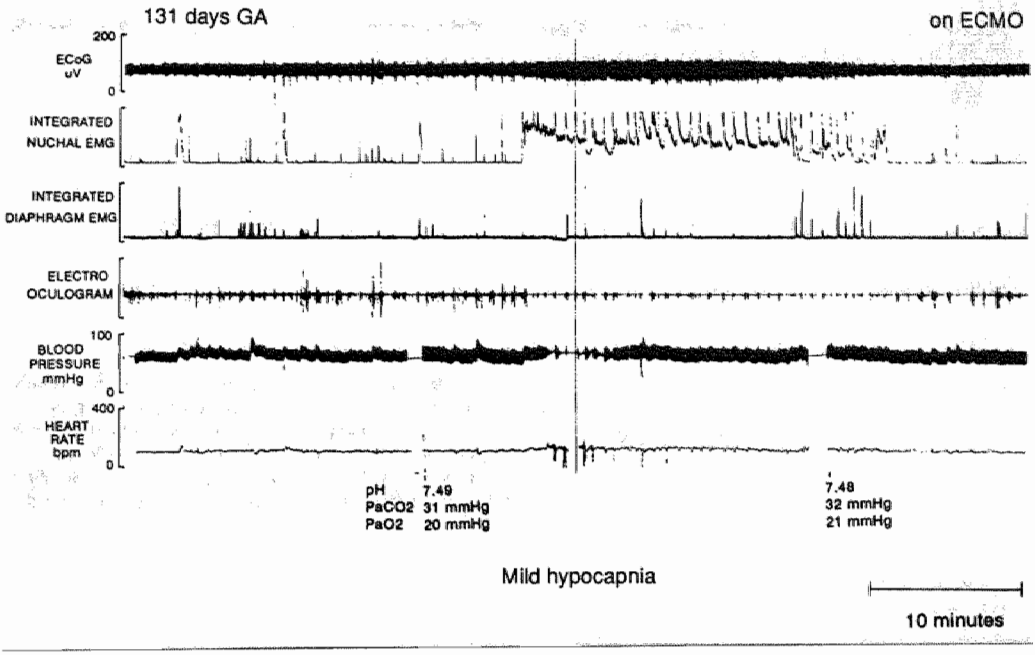

Figure 4.1 Intrauterine recording of a fetus of 131 days of gestation connected to the ECMO system 3 days after surgery. Tracings are from the top: ECoG activity, integrated nuchal EMG, integrated diaphragm EMG, electro-oculogram, blood pressure and heart rate. Blood gas and pH samples were taken at the indicated time. During mild hypocapnia there were almost no breathing movements present during LV ECoG.

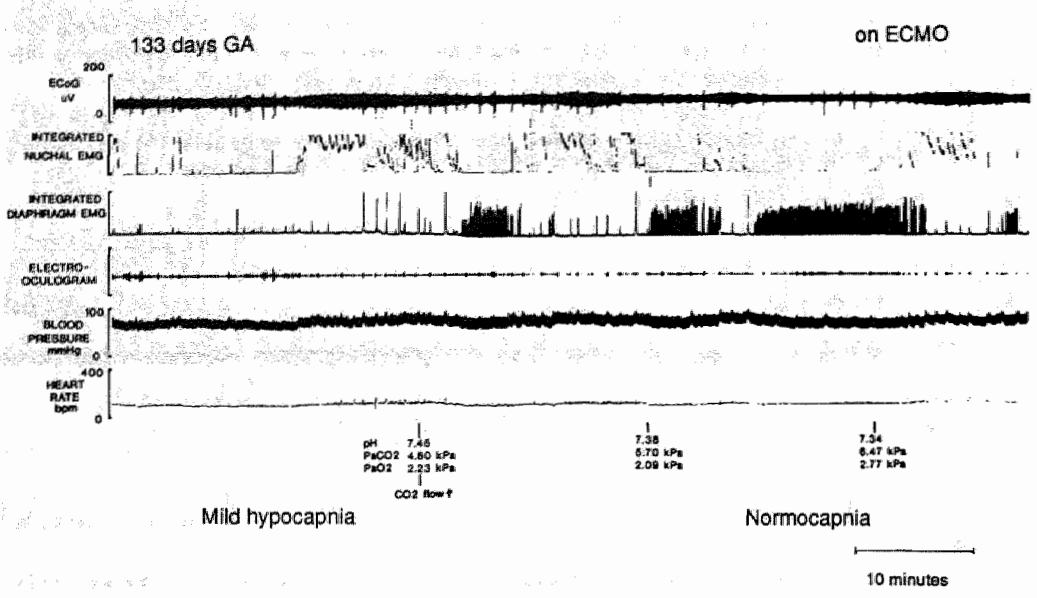

Figure 4.2 Intrauterine recording of a fetus of 133 days of gestation connected to the ECMO system 4 days after surgery. Tracings are from the top: ECoG activity, integrated nuchal EMG, integrated diaphragm EMG, electro-oculogram, blood pressure, heart rate. Blood gas and pH samples were taken at the indicated time. During mild hypocapnia there were no breathing movements present during LV ECoG. After increasing CO_2 flow to the membrane to obtain fetal normocapnia, breathing movements reappeared and were associated with LV ECoG.

Discussion

The use of ECMO in utero enabled us to study the effect of mild hypocapnia during normoxia on fetal behavioral activity in fetuses of 130-134 days gestation, an age at which ECoG was differentiated. The incidence and the length of periods of fetal breathing movements decreased significantly during periods of mild hypocapnia. However, there was no change in the incidence or duration of periods of LV ECoG. Nuchal EMG and rapid eye movements were normal during mild hypocapnia periods. This suggests that lowering $PaCO_2$ reduces the drive for fetal breathing activity.

The use of an ECMO system could by itself introduce new variables and influence fetal behavioral activity. However, we did not observe statistical differences in the incidence or duration of periods of fetal breathing movements or LV ECoG activity before or during ECMO. The fetuses showed no abnormal behavioral activity during the baseline periods on ECMO, as LV ECoG activity remained associated with rapid eye movements and fetal breathing movements, and HV ECoG remained associated with nuchal muscle activity. Since these parameters (fetal

breathing movements and fetal behavior) are generally used to define the 'normality' of the preparation we do not consider that the use of extracorporeal circulation by itself influenced the results.

In our experiments there was no change in the incidence or duration of periods of LV ECoG activity during hypocapnia. Rapid eye movements remained associated with LV ECoG activity and nuchal EMG activity remained associated with HV ECoG activity. Despite the fact that LV ECoG did not change, the incidence and the length of periods of breathing movements and the incidence of fetal breathing movements during LV ECoG decreased significantly during hypocapnia. This suggests that fetal breathing activity can be altered independently of other aspects of behavioral states. Although we found a significant reduction in breathing activity it was not totally abolished. Presumably this was because we used only mild hypocapnia. In adult cats it has been reported that graded reduction in $PaCO_2$ to as low as 0.5 kPa caused a graded reduction of breathing activity to the point where breathing became irregular (Berkenbosch et al. 1984). In the fetus, we do not know whether there is a threshold below 4 kPa at which breathing activity becomes no longer present.

Previous studies in animals and man (Boddy et al. 1974, Connors et al. 1988, Marsál et al. 1979) reported a decrease of fetal breathing activity during hypocapnia. In human studies (Connors et al. 1988, Marsál et al. 1979) fetal hypocapnia was obtained by maternal hyperventilation which limited the duration of the experiments to 15 min (Connors et al. 1988) or less (Marsál et al. 1979). Maternal hyperventilation and hypocapnia present several problems, in particular the decrease in uterine and in umbilical blood flow (Oakes et al. 1976). This can result in fetal hypoxemia and acidosis (Oakes et al. 1976) although no fetal blood samples were taken in human studies. In the sheep fetus Boddy et al. (1974) reported a decrease in incidence of breathing movements during hypocapnia obtained by maternal hyperventilation for an unspecified period in 2 normoxic fetal lambs. We have now extended these observations by maintaining fetal hypocapnia for several hours under conditions where the fetus remained normoxemic.

Whilst we maintained fetal PaO_2 constant during hypocapnia, it is possible that, under these hypocapnic conditions cerebral blood flow will decrease ca. 25% (Rosenberg et al. 1982). The O_2 dissociation curve will shift to the left (Bohr-effect) and pyruvate and lactate concentrations may increase in brain tissue and CSF (Rosenberg 1988, Siesjo & Ingvar 1986). Therefore, hypocapnia may be associated with *central* hypoxemia. There are several reasons why we do not think that this was likely to have been the cause of the reduction in breathing which we observed. First, in our experiments arterial hypocapnia was only mild (mean delta $PaCO_2$ of 1.44 kPa). In piglets a decrease in cerebral blood flow only occurred during severe hypocapnia ($PaCO_2 < 2$ kPa) although even then there was maintenance of the flow to the brain stem (Hansen et al. 1984). Furthermore, maintenance of oxygen consumption occurs during hypocapnia due to an increase in cerebral oxygen extraction (Rosenberg 1988). Lastly, during hypoxemia, breathing activity, nuchal EMG activity

and rapid eye movements are inhibited (Boddy et al. 1974, Koos et al. 1987a, Woudstra et al. 1990): but in our experiments, beside the decrease in the incidence of breathing movements, we did not see such an inhibition of rapid eye movements or nuchal EMG activity. Therefore, we conclude that the effects on breathing were not due to central hypoxemia but to mild hypocapnia and alkalosis.

After mild hypocapnia periods, when fetal $PaCO_2$ returned to baseline values, breathing activity reappeared within 4 min. It remained periodic but its incidence was increased compared to the normocapnia periods. Walker et al. (1986) observed a significant increase in breathing activity and even breathing activity during HV ECoG (Walker et al. 1986) after a period of 8 hours of maternal hyperthermia which produced fetal hypocapnia. Such prolonged hypocapnia will produce a reduction in brain bicarbonate concentration, and therefore when $PaCO_2$ is returned to normal a relative central acidosis will result (Fencle 1986). It is known that lowering CSF pH or producing central acidosis using NH_4Cl stimulates fetal breathing activity (Hohimer et al. 1983, Molteni et al. 1980).

In conclusion, the level of $PaCO_2$ is an important determinant of the incidence of breathing activity in utero. This incidence can be changed independently of that of behavioral states. The mechanism by which CO_2 determines the activity of the respiratory network in the fetal brainstem has to be determined, but clearly it is a fundamental determinant of the presence of fetal breathing activity.

Chapter 5

The effect of hypercapnia and hypercapnia associated with central cooling on behavior in unanesthetized fetal lambs

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Submitted

Abstract

In utero breathing activity is periodically present and it must become continuous at birth. We investigated the effect of hypercapnia and of hypercapnia combined with central cooling on fetal breathing periodicity in lambs, using an ECMO system to control fetal blood gases and fetal temperature in 7 chronically instrumented fetal lambs of 131-134 days gestation. During fetal hypercapnia ($PaCO_2$ 7.39 ± 0.15 kPa) frequency, amplitude and incidence of fetal breathing movements during LV ECoG increased significantly compared to isocapnic control on ECMO but it remained absent during HV ECoG. During hypercapnia associated with central cooling ($PaCO_2$ 7.90 ± 0.13 kPa, temperature decrease 2.1 ^{0}C) there were similar changes in fetal breathing movements during LV ECoG. However, in 4 out of 7 fetuses fetal breathing movements continued throughout HV ECoG. Hypercapnia associated with central cooling can thus override the inhibitory effects of HV ECoG on fetal breathing movements. This may be due to changes in CO_2 sensitivity produced by an increase in afferent input to the central nervous system.

Introduction

In utero, after maturation of electrocortical activity, breathing activity is inhibited during HV ECoG (Dawes et al. 1972, Dawes et al. 1983). This inhibition must be overridden at birth to allow the establishment of continuous breathing. The mechanisms involved in the initiation of continuous breathing at birth are not completely understood. Fetal hypercapnia, obtained by increasing maternal $PaCO_2$ produces an increase in incidence, amplitude and frequency of fetal breathing movements only during LV ECoG (Boddy et al. 1974, Chapman et al. 1980, Ritchie & Lakhani 1980, Bowes et al. 1981b, Dawes et al. 1982, Connors et al. 1988, Connors et al. 1989). It is known that the level of $PaCO_2$ increases after cord occlusion at birth and an increase in $PaCO_2$ it is important for the initiation of breathing (Blanco et al. 1987b, Berger et al. 1990).

Mechanisms involved in the inhibition of breathing during HV ECoG are not known. It is possible that changes in CO_2 sensitivity could play a role. This idea could be supported by the observed continuous breathing activity obtained by a decrease in cutaneous fetal temperature (Gluckman et al. 1983). Johnston and Gluckman (1989) described continuous breathing activity during hypercapnia but not during normocapnia in fetal lambs with lesions in the rostral lateral pons. It could be speculated that these lesions liberated the respiratory centres from inhibitory influences present during HV ECoG. These observations support the idea that a decrease in sensitivity to CO_2 could occur during HV ECoG in intact animals resulting in the loss of respiratory drive and apnea (Johnston & Gluckman 1989). If this mechanism exists, it could play a role at birth

when there is a combination of an increased afferent input, a decrease in temperature and hypercapnia.

A previous study showed that a decrease in core temperature produced by an internal coil failed to produce continuous breathing (Gluckman et al. 1983). This would not support our proposal but there are a number of other factors which could have been responsible for these results. In that study there was no change in fetal $PaCO_2$ and the cold internal coil passing the pharynx and larynx could have stimulated the upper airway sensitive receptors inhibiting breathing (Gluckman et al. 1983). We decided to use a different approach by using an extra corporeal membrane oxygenation system with which blood gases and blood temperature can be changed. We tested the hypothesis that the association of hypercapnia with central cooling, obtained by cooling the blood of the circuit, could override the central inhibition during HV ECoG resulting in continuous breathing activity. This might be an important mechanism involved in the initiation of continuous breathing at birth.

Materials and methods

Experiments were performed on unanesthetized chronically instrumented fetal sheep in utero (Chapter 3).

Recordings were obtained before connecting the animal to ECMO, during control periods on ECMO, during hypercapnia periods and during periods of hypercapnia associated with central cooling. Recordings on ECMO were taken at least 1 hour after starting ECMO perfusion. Fetal hypercapnia was obtained by increasing the CO_2 concentration of the gasflow to the membrane lung. Hypercapnia was defined as a fetal $PaCO_2$ at least 1 kPa higher than baseline. The core temperature of the fetus was decreased by 1-3 °C by decreasing circuit blood temperature. This was obtained by turning off the heating element of the box containing the circuit and placing ice around the membrane lung and in contact with the circuit. It took 20-30 min to decrease fetal central temperature by 1 °C at a pump flow of 200 ml/min. Whenever fetal $PaCO_2$ did not increase significantly from control CO_2-flow to the membrane lung was increased to obtain the desired $PaCO_2$ level. The moment when the experimental conditions periods of hypercapnia associated with central cooling were reached ($PaCO_2$ increased 1 kPa, central temperature decreased 1 ^{0}C) was considered the starting point of the experiments.

The protocol was divided into 3 periods during ECMO a) control periods, b) hypercapnia periods, then returning to normocapnia and c) hypercapnia associated with central cooling periods. There was a minimum control period on ECMO of at least 2 hours. Hypercapnia and hypercapnia & central cooling experimental periods were at least 1 hour (except in one fetus in which the experiment was 30 min). Fetal blood gases were taken approximately every 15 min during a control or experimental period and were averaged. The incidence per hour and length of periods of LV ECoG activity were analyzed. Furthermore, the incidence of fetal breathing movements per hour, the incidence of fetal

breathing movements during LV ECoG, frequency and amplitude of fetal breathing movements were analyzed. Frequency of fetal breathing movements per minute was analyzed by counting all breaths after replaying the tape at a higher paper speed. The amplitude was analyzed by measuring the area under the curve of the integrated EMG activity of the diaphragm (Quantimed 570, Leica). The area under the integrated nuchal EMG activity was determined in the same way as the area of the fetal breathing movements which resulted in percentage. The amplitude of breathing movements and the amount of nuchal muscle activity were expressed as a percentage of the mean breath amplitude and the mean nuchal muscle activity during control periods on ECMO (100%) for each fetus. Mean arterial blood pressure and heart rate were analyzed every 10 min and averaged over the experimental periods. Fetal blood gases and pH were analyzed from fetal arterial blood samples with a Radiometer ABL3 at 39.5 °C. During cooling periods fetal blood gases were corrected for fetal central temperature.

All data are reported as means ± SEM. Wilcoxon signed rank test was used for statistical comparison of variables between hypercapnia and control periods and between hypercapnia associated with cooling and hypercapnia periods.

Results

Experiments were performed on 7 fetal lambs, at gestational age 131-134 days.

Baseline recordings prior to connection to the ECMO system were obtained at least 48 hours (55-70 hours) after surgery for a duration of at least 11 hours. Fetal blood gases and pH were prior to connection to the ECMO system: pH 7.36 ± 0.01, $PaCO_2$ 5.84 ± 0.14 kPa, PaO_2 3.15 ± 0.23 kPa. Mean arterial blood pressure was 67.7 ± 3 mmHg, and heart rate was 163.8 ± 8 bpm. Nuchal EMG was always associated with HV ECoG. Rapid eye movements (n=5) were always associated with LV ECoG and fetal breathing movements. During the baseline LV ECoG occurred 52.5 ± 2.0% of the time. Fetal breathing movements were present 36.3 ± 3.0% of the total time and 69.4 ± 5.4% during LV ECoG.

Control periods on ECMO

On the third day post-surgery the fetuses were connected to the ECMO system.

In table 5.1 physiological variables are reported. Nuchal EMG activity was associated with HV ECoG; rapid eye movements were associated with LV ECoG and fetal breathing movements.

Table 5.1 **The effect of hypercapnia and hypercapnia with central cooling on behavior, blood pressure, heart rate, pH and blood gases of the fetus.**

Physiologic variables	*Control on ECMO*	*Hypercapnia on ECMO*	*Hypercapnia & central cooling on ECMO*
pH	7.36 ± 0.01	7.31 ± 0.01[1]	7.26 ± 0.01[2]
$PaCO_2$ (kPa)	6.18 ± 0.11	7.39 ± 0.15[1]	7.90 ± 0.13
PaO_2 (kPa)	4.64 ± 0.38	5.17 ± 0.7	4.81 ± 0.84
LV ECoG (%)	51.0 ± 2.6	49.8 ± 3.4	48.4 ± 9.7
LV ECoG, LP (min)	18.6 ± 1.8	17.2 ± 2.3	16.9 ± 3.4
FBM/HV ECoG	0 exp	0 exp	4/7 exp
FBM/LV ECoG (%)	83.5 ± 2.5	89.8 ± 2.4[1]	92.1 ± 4.5
Frequency FBM (breaths/min)	40.6 ± 8.1	47.5 ± 7.6[1]	25.3 ± 6.8[2]
Amplitude FBM (%)	100	135.6 ± 11.8[1]	132.8 ± 13.9
Area nuchal EMG (%)	100	95.0 ± 16.5	191.0 ± 30.6[2]
Blood pressure (mmHg)	69 ± 3.9	68 ± 2.6	75 ± 2.9[2]
Heart rate (bpm)	172 ± 10.9	168 ± 8.9	210 ± 9.5[2]

Means ± SEM of fetal blood gases and pH, incidence and length of periods of LV ECoG and the incidence of fetal breathing movements during LV ECoG (FBM/LV ECoG), frequency and amplitude of fetal breathing movements, area of nuchal EMG, blood pressure and heart rate. Furthermore the presence of fetal breathing movements during HV ECoG. (LP; length of period, FBM; fetal breathing movements, %; percentage of the time present or percentage increase of amplitude of fetal breathing movements or area of nuchal muscle activity, exp; experiments, [1] $p<0.05$, compared to control on ECMO, [2] $p<0.05$, compared to hypercapnia on ECMO).

Hypercapnia periods on ECMO

Nuchal EMG activity was present and associated with HV ECoG, the area of nuchal muscle activity was not different from control on ECMO. Compared to control periods on ECMO there was a significant increase in amplitude, frequency and incidence of fetal breathing movements during LV ECoG. However, breathing movements remained periodically present associated with rapid eye movements and LV ECoG (see figure 5.1).

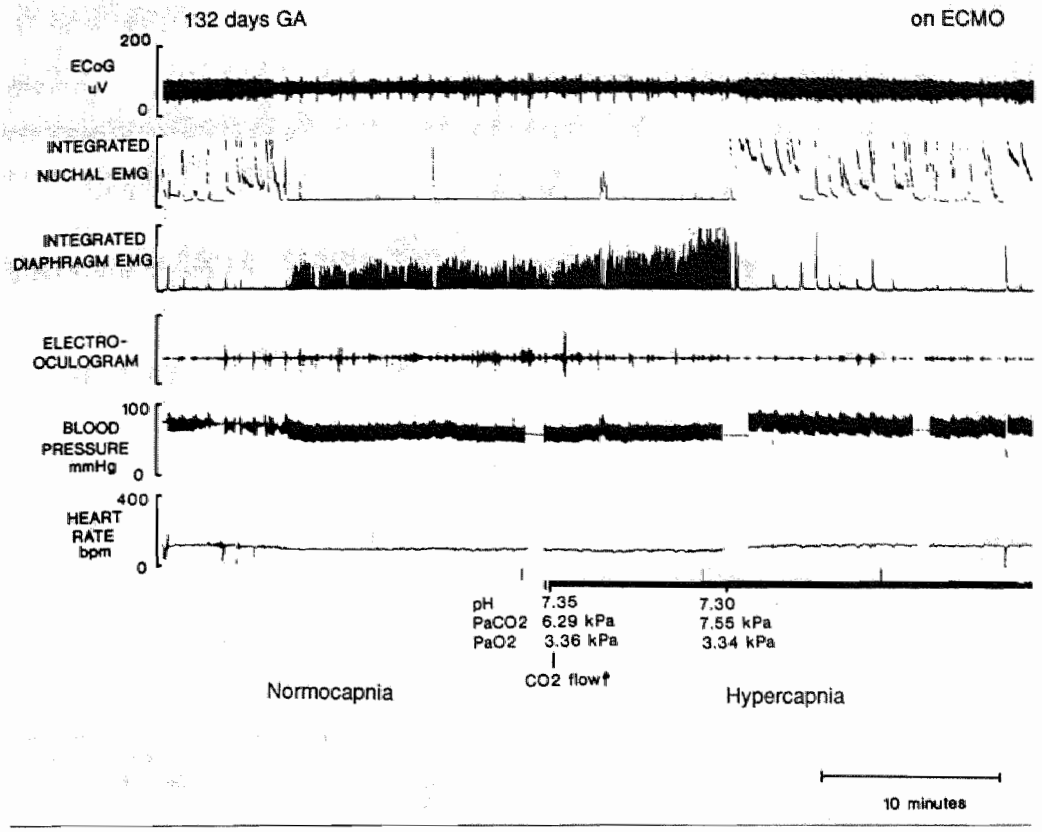

Figure 5.1 Intrauterine recording of a fetus at 132 days gestation, 3 days after surgery. Tracings are from the top: electrocortical activity, integrated nuchal EMG, integrated diaphragm EMG, electro oculogram, blood pressure and heart rate. Blood gas and pH samples were taken at the indicated times, CO_2-flow was increased at the indicated time. Note that fetal breathing movements were only present during LV ECoG, even during hypercapnia and associated with rapid eye movenents. Nuchal muscle activity was present during HV ECoG.

Hypercapnia & central cooling on ECMO

In 5 out of 7 experiments fetal $PaCO_2$ did not increase significantly after 20-30 min while fetal central temperature was decreased by 1 ^{0}C. Fetal breathing remained present only during LV ECoG despite fetal central temperature being decreased by 1 ^{0}C. In these experiments fetal hypercapnia was obtained by increasing CO_2-flow to the membrane lung. In 2 out of 7 experiments fetal $PaCO_2$ increased to a hypercapnic level without increasing CO_2-flow to the membrane lung.

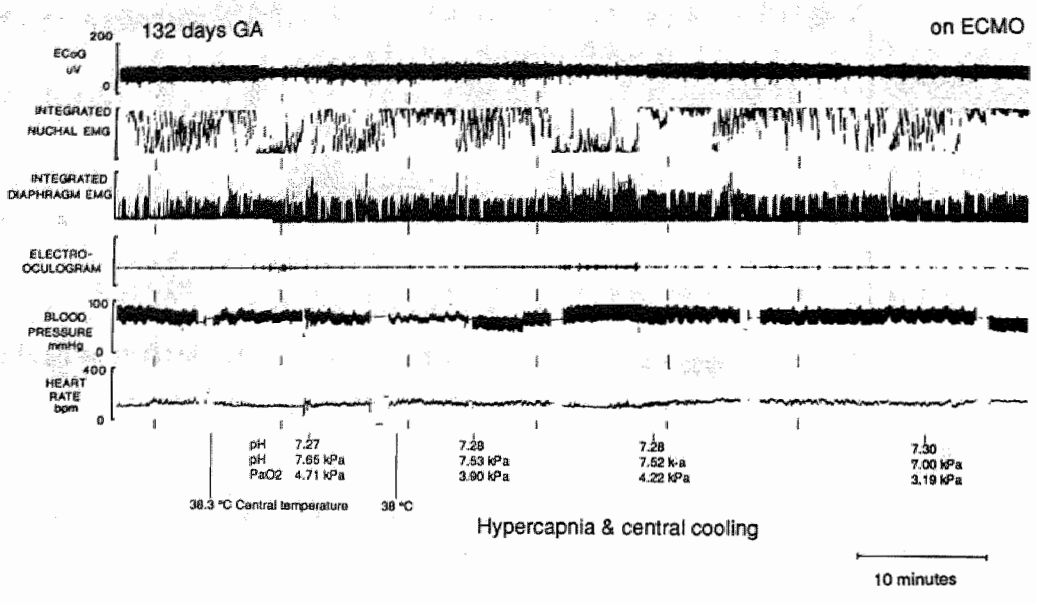

Figure 5.2 Intrauterine recording of a fetus at 132 days gestation, 4 days after surgery. Tracings are from the top: electrocortical activity, integrated nuchal EMG, integrated diaphragm EMG, electro oculogram, blood pressure and heart rate. Blood gas and pH samples were taken at the indicated times. Note that fetal breathing movements were still present during HV ECoG. Nuchal muscle activity was continuously present. Both were still modulated by electrocortical activity.

In the experiments where hypercapnia was associated with central cooling, central temperature had decreased by a mean of 2.1 °C and fetal $PaCO_2$ increased to 7.90 ± 0.13 kPa. Nuchal muscle activity was present during both ECoG states, increased during HV ECoG and still present during LV ECoG but decreased in amplitude (see figure 5.2). The amount of nuchal muscle activity was 191.0 ± 30.6% ($p< 0.05$ compared to hypercapnia on ECMO). Rapid eye movements were present during LV ECoG. In 3 out of 7 fetal lambs breathing activity remained associated with LV ECoG. Breathing activity was present continuously in 4 out of 7 fetal lambs. In these experiments breathing movements were both present during HV ECoG and LV ECoG (see figure 5.2). During hypercapnia associated with central cooling frequency of fetal breathing movements decreased significantly compared to hypercapnia alone. However, there was no change in amplitude and breathing movements were still modulated by electrocortical activity resulting in a lower frequency during HV ECoG than during LV ECoG.

Discussion

The incidence, frequency and amplitude of fetal breathing movements during LV ECoG increased significantly during hypercapnia as expected (Boddy et al. 1974). There was no change in incidence or length of periods of LV ECoG either during hypercapnia or during hypercapnia associated with central cooling. During hypercapnia associated with central cooling there was an increase in nuchal muscle activity, blood pressure, heart rate, a decrease in frequency of fetal breathing movements but no change in amplitude. Furthermore, breathing activity became continuous in 4 out of 7 fetal lambs. These results showed that the association of hypercapnia with central cooling could override the normally present inhibition of fetal breathing movements during HV ECoG resulting in continuous breathing activity.

As previously discussed ECMO could introduce new variables which could change fetal behavior. However, during ECMO there was normal fetal behaviour expressed by a normal incidence of LV ECoG and fetal breathing movements, rapid eye movements remained associated with LV ECoG and nuchal muscle activity with HV ECoG. It is therefore unlikely that the use of extracorporeal circulation by itself could have influenced the results. The originality of our work was that we could control fetal temperature and blood gases directly independently of the ewe or the placenta.

In utero, after maturation of electrocortical activity fetal breathing movements and rapid eye movements are only present during LV ECoG and nuchal EMG activity is present during HV ECoG and absent during LV ECoG (Dawes et al. 1972, Clewlow et al. 1983, Ioffe et al. 1987). Hypercapnia is known to stimulate fetal breathing movements (Boddy et al. 1974, Chapman et al. 1980, Ritchie & Lakhani 1980, Bowes et al. 1981b, Dawes et al 1982, Jansen et al. 1982, Clewlow et al. 1983, Rigatto et al. 1988). Fetal hypercapnia obtained with ECMO produced an increase in frequency, amplitude and incidence of fetal breathing movements during LV ECoG which is in agreement with earlier studies. During these hypercapnia periods there was no change in fetal behavioral activity, since there was no change in incidence or length of periods of LV ECoG, eye movements remained associated with LV ECoG and nuchal EMG with HV ECoG. At variance with previous reported experiments an increase in incidence of LV ECoG during fetal hypercapnia obtained by ECMO did not occur. The increase in incidence of LV ECoG during maternal hypercapnia could be due to the release of substances produced by the placenta or the ewe during maternal hypercapnia. During hypercapnia associated with central cooling there was an increase in amplitude compared to control and a decrease in frequency. The decrease in frequency might be related to the decrease in oxygen consumption during cooling periods. Breathing movements were still modulated by electrocortical activity since the frequency of breathing movements was lower during HV ECoG than during LV ECoG. This might be due to the difference of control during LV ECoG and during HV ECoG since

breathing movements are metabolically controlled during HV ECoG, behaviorally controlled during LV ECoG (Phillipson et al. 1986).

Hypercapnia alone was not sufficient to initiate respiratory activity during HV ECoG, as reported earlier (Walker et al. 1976, Dawes et al. 1982, Jansen et al. 1982, Rigatto et al. 1988, Faucher et al. 1991). The mechanism for this inhibition during HV ECoG is not very well understood. In utero breathing activity seems to be much more dependent on peripheral input during HV ECoG and this mechanism could be involved in fetal apnea during HV ECoG. This was supported by experiments where continuous breathing activity was induced by electrical stimulation of the sciatic nerve (Moss & Scarpelli 1979) or by decreasing cutaneous fetal temperature (Gluckman et al. 1983). Central mechanisms also play a role since fetuses with a brain stem transection or with small lesions in the rostral lateral pons can show fetal breathing movements during HV ECoG (Dawes et al. 1983, Johnston & Gluckman 1989). In the latter, continuous breathing only occurred during hypercapnia which suggested that the lesions interfered with cortical inhibitory influences changing the sensitivity for CO_2 within the brain stem. In our experiments in 4 out of 7 fetuses breathing activity became present continuously when hypercapnia was associated with central cooling. This suggests that extra peripheral afferent input produced by central cooling might have changed central inhibitory processes allowing an increase in the sensitivity for CO_2. We cannot explain why breathing did not become continuously in all experiments, since there was no difference in the decrease of central temperature, in the amount of nuchal muscle activity or in the level of fetal $PaCO_2$. It could be speculated that the presence of continuous breathing is due to subtle changes in CO_2 sensitivity produced by increased afferent input. The amount of afferent input was in this work not quantified more than by measuring the amount of nuchal muscle activity and temperature changes. Therefore, it is impossible to know whether the stimulus was similar. The quantification of afferent input is difficult and it would require direct recording of brain stem reticular substance. Subtle changes in ECoG or states were also not possible to observe due to the characteristics of our recording technique. Previous attempts to induce continuous breathing in utero by decreasing central temperature with an internal coil passing the pharynx, the larynx, oesophagus, and a loop in the stomach, were unsuccessful (Gluckman et al. 1983). It is possible that upper airway receptors were stimulated by the low temperature of the coil passing the upper airway and inhibited breathing activity. Another problem could have been that in those experiments the fetuses remained normocapnic. In our experiments breathing activity remained absent during HV ECoG when fetal $PaCO_2$ did not increase during central cooling. Further indication that fetal continuous breathing might be determined by the level of fetal $PaCO_2$ is given by experiments where continuous breathing produced by hypercapnia associated with central cooling was not sustained when the fetal $PaCO_2$ was decreased to normocapnic or hypocapnic level before birth (Kuipers et al. 1994c). In our study the association between central cooling and hypercapnia was essential for producing continuous breathing

activity since neither could produce it independently. Further evidence of the influence of temperature on CO_2 sensitivity was provided by experiments done on newborn animals which showed a decreased respiratory response to CO_2 in a warmer environment (Wanatabe et al. 1993, Malcolm & Henderson-Smart 1994).

Central hypothermia did not change the fetal ECoG pattern. Nuchal muscle activity was increased but still modulated by LV ECoG. We assumed that this increased activity of the nuchal muscles reflected shivering activity since this enhanced activity was only present during the cooling periods (Gluckman et al. 1983).

There was a significant increase in blood pressure and heart rate during hypercapnia associated with central cooling, probably due to an increase of catecholamine levels (Sidi et al. 1983, Gunn et al. 1985).

In summary, direct fetal hypercapnia resulted in a significant increase in amplitude, frequency and incidence of fetal breathing movements during LV ECoG but no increase in incidence of LV ECoG. Central cooling associated with hypercapnia could override the inhibition during HV ECoG resulting in continuous breathing. It might be speculated that cooling could have increased afferent input to the brain stem changing the CO_2 sensitivity and overriding the inhibition during HV ECoG. This mechanism could contribute to the initiation of continuous breathing at birth.

Chapter 6

The effect of maternal hypoxemia on behavior in unanesthetized normoxic or mildly hyperoxic fetal lambs

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Abstract

To determine if hypoxemia inhibits fetal activity by substances from the mother or placenta, six fetal lambs were chronically instrumented at 128-132 days gestation for ECMO. Severe maternal hypoxemia (PaO_2 decreased to 6.00 ± 0.60 kPa) was produced while fetal PaO_2 was maintained normoxic or mildly hyperoxic using ECMO. The incidence of fetal breathing movements was 34.8 ± 3.1% during baseline before ECMO, 36.8 ± 3.4% during baseline on ECMO, and 21.4 ± 3.5% ($p< 0.05$ compared to baseline on ECMO) during maternal hypoxemia. The duration of periods of breathing was 9.8 ± 1.2 min before ECMO, 9.3 ± 1.1 min on ECMO and 10.5 ± 1.7 min (ns) during maternal hypoxemia. In 7 of 14 maternal hypoxemia experiments breathing activity stopped too late (7-23 min) to be attributed to maternal hypoxemia. Fetal ECoG activity (ns), nuchal muscle activity (ns) and rapid eye movements were present as normal before and on ECMO and during maternal hypoxemia and fetal blood pressure or heart rate did not change.

We conclude that the inhibition of fetal activity during maternal hypoxemia does not seem to be mediated by release of factors from the maternal side of the placenta or the ewe.

Introduction

In contrast to after birth acute hypoxemia inhibits fetal activity. This is seen as a decrease in breathing activity (Blanco et al. 1983b, Bocking et al. 1988, Boddy et al. 1974, Koos et al. 1987a), in limb movements and nuchal muscle activity (Blanco et al. 1983b, Bocking et al. 1988, Koos et al. 1987a, Natale et al. 1981, Woudstra et al. 1990), in rapid eye movements (Bocking et al. 1988, Koos et al. 1987a), in incidence and frequency of swallowing (Sherman et al. 1991) and in the magnitude of hind-limb reflexes (Blanco et al. 1983b). The length of periods and incidence of ECoG activity may or may not be affected by acute hypoxemia (Adamson et al. 1984, Boddy et al. 1974). The inhibitory response to hypoxemia occurs rapidly: for example, fetal breathing movements are inhibited within 4 min (Clewlow et al. 1983, Koos et al. 1987a), hind-limb spinal reflexes are reduced within 5 min (Blanco et al. 1983b). Cardiovascular responses, seen as the increase in arterial blood pressure and the decrease in heart rate, occur within 3-4 min (Blanco et al. 1983b).

Several studies have implicated an area in the upper pons of the fetal or neonatal brainstem in mediating the fetal response to hypoxemia. These studies employed a range of techniques, including brainstem transection (Dawes et al. 1983), focal cooling (Moore et al. 1991), the placement of lesions in the lateral pons (Gluckman & Johnston 1987), electrical stimulation or recording from neurons in the lateral pons (Coles et al. 1989). These studies have been interpretated as showing that an active

central inhibition is triggered by a decrease in brain tissue oxygenation. However, the mechanisms involved in this response and the nature and location of the chemosensitive area which senses the change in oxygen availability are unknown.

In the majority of the studies the effects of short term acute hypoxemia on fetal activity were examined. Fetal hypoxemia was induced by having the ewe breathe a hypoxic gas mixture or by reducing uterine blood flow (Bocking & Harding 1986, Boddy et al. 1974). Alternatively, a reduction in O_2 availibility by means of fetal methemoglobinemia, fetal carboxyhemoglobinemia or fetal anemia has also been found to produce cessation of fetal breathing activity (Koos et al. 1988b, Koos et al. 1990a, Koos et al. 1987b). These studies show that a fall in tissue oxygenation, rather than a decrease in PaO_2, is responsible for producing the cessation of fetal breathing activity. However the site at which this fall in oxygenation is detected is not apparent from these studies. For example, they cannot rule out the possibility that release of modulatory substances from the fetal side of the placenta played a part in mediating the response. Indeed, it is known that O_2-deficient tissues, such as the brain, heart and placenta produce substances (e.g. prostaglandins, adenosine and beta-endorphins) which could modulate fetal activity (Kitterman et al. 1983, Koos et al 1992, Slegel et al. 1988). These substances could mediate their effects via the lateral pons, alternatively, it may be that the lateral pons must be functionally intact for their effects to be seen. This would explain the observations that destruction of the brain stem alters the response (Koos et al. 1992).

This paper presents the results of studies aimed at examining the possibility that a substance produced by the mother or the maternal side of the placenta during hypoxemia produces an inhibitory effect on fetal behavior. In order to approach this problem we used an ECMO system in chronically instrumented fetal lambs. This allowed us to keep the fetus normoxic or mildly hyperoxic while the ewe was exposed to acute hypoxemia by breathing a hypoxic gas mixture.

Materials and methods

Experiments were performed on unanesthetized chronically instrumented fetal lambs in utero (Chapter 3).

Fetal blood gases and pH, the incidence per hour and the length of periods of LV ECoG activity, the incidence of nuchal muscle activity during HV ECoG, the incidence per hour and length of periods of fetal breathing movements were measured a) during periods before connection to the ECMO system, b) during baseline periods on ECMO and c) when the ewe was exposed to acute hypoxemia and the fetus remained normoxic or mildly hyperoxic. Maternal hypoxemia was obtained by having the ewe breathe through a bag supplied with a gas mixture of 9% O_2, 3% CO_2 and 88% N_2 (40 l/min). During this time the fetus was kept normoxic or mildly hyperoxic using the ECMO system. Maternal hypoxemia was initiated when the fetus showed breathing activity for at

least one minute. The incidence of fetal breathing movements and LV ECoG (min/hr) was only analyzed for experiments with a duration of more than 30 min. Mean blood pressure and heart rate were measured every 10 min during baseline recordings before connection to the ECMO system, during baseline recordings on ECMO and every 5 min during maternal hypoxemia experiments. Amniotic pressure was not subtracted from the blood pressure.

The Friedman and Wilcoxon signed rank test were used for statistical comparison of the incidence of nuchal EMG activity during HV ECoG, the incidence and length of periods of LV ECoG, the incidence and length of periods of fetal breathing movements, blood pressure and heart rate between baseline periods before connection to the ECMO system and the baseline periods on ECMO, and between the latter and periods of maternal hypoxemia. The median was used to express the time between experiments and the duration of maternal hypoxemia experiments.

Results

Experiments were performed on 6 fetal lambs, at 131-135 days gestational age.

Baseline recordings before connecting the fetus to the ECMO system

Baseline recordings were obtained 55 to 70 hours after the operation, the duration of these recordings being 11 to 28 hours. Fetal blood gases and pH just before connection to the ECMO system were: pH 7.35 ± 0.01, $PaCO_2$ 5.92 ± 0.20 kPa, PaO_2 2.28 ± 0.22 kPa. Mean arterial blood pressure was 66 ± 4 mmHg (unreferenced), heart rate was 157 ± 10 bpm.

Nuchal EMG activity was present 81.2 ± 5.7% of the time during HV ECoG activity. Rapid eye movements (2 fetuses) were associated with LV ECoG activity and fetal breathing movements. Fetal breathing movements were only present during LV ECoG. LV ECoG activity occurred 52.5 ± 2.0% of the time and the mean length of the LV ECoG periods was 13.5 ± 0.8 min (table 6.1). Fetal breathing activity was present 34.8 ± 3.1% of the time, the mean length of the fetal breathing periods being 9.8 ± 1.2 min (table 6.1).

Table 6.1 **Incidence and length of periods of fetal breathing movements and LV ECoG activity during baseline recordings before connection to the ECMO system, during baseline recordings after connection to the ECMO system and during maternal hypoxemia experiments.**

Physiologic variables	*Baseline before ECMO*	*Baseline on ECMO*	*Maternal hypoxemia*
LV ECoG (%)	52.5 ± 2.0	47.5 ± 2.0	44.3 ± 7.4
LV ECoG, LP (min)	13.5 ± 0.8	14.3 ± 1.5	17.4 ± 2.3
FBM (%)	34.8 ± 3.1	36.8 ± 3.4	21.4 ± 3.5[1]
FBM, LP (min)	9.8 ± 1.2	9.3 ± 1.1	10.5 ± 1.7

Means ± SEM of incidence and length of periods of LV ECoG and fetal breathing movements. (LP; length of period, FBM; fetal breathing movements, %; percentage of the time present, [1] $p<0.05$, compared to baseline on ECMO).

Baseline recordings after connecting the fetus to the ECMO system

On the third day post-surgery the fetuses were connected to the ECMO system. Baseline recordings were obtained in the different fetuses from one hour after connection to the ECMO system. Fetal pH and blood gases during these baseline recordings on the ECMO system were: pH 7.37 ± 0.02, $PaCO_2$ 6.16 ± 0.18 kPa, PaO_2 3.92 ± 0.36 kPa (PaO_2, $p<0.05$ compared to before connection to the ECMO system). Mean arterial blood pressure was 74 ± 5 mmHg, heart rate was 171 ± 13 bpm. Nuchal EMG activity was present 88.2 ± 4.8% of the time during HV ECoG activity. Rapid eye movements (n=2) were always associated with LV ECoG activity and fetal breathing movements. Fetal breathing movements were only present during LV ECoG. The incidence of LV ECoG activity was 47.5 ± 2.0% and the mean length of the LV ECoG periods was 14.3 ± 1.5 min (table 6.1). Breathing activity was present 36.8 ± 3.4% of the time, the mean length of the fetal breathing periods being 9.3 ± 1.1 min (table 6.1).

Table 6.2 **Fetal and maternal blood gases and pH before, at 5 min and at 30 min of maternal hypoxemia experiments.**

	before MH	*5' MH*	*30' MH*
F-pH	7.36 ± 0.01	7.36 ± 0.01	7.36 ± 0.01
F-$PaCO_2$ (kPa)	6.20 ± 0.22	6.18 ± 0.18	5.90 ± 0.35
F-PaO_2 (kPa)	5.37 ± 0.54	4.18 ± 0.47[1]	4.36 ± 0.55
M-pH	7.49 ± 0.01	7.50 ± 0.03	7.50 ± 0.03
M-$PaCO_2$ (kPa)	3.98 ± 0.20	4.03 ± 0.19	3.96 ± 0.18
M-PaO_2 (kPa)	13.58 ± 0.70	6.00 ± 0.60[1]	5.63 ± 0.77[1]

Means ± SEM of fetal (F) en maternal (M) blood gases and pH. ([1] $p<0.05$, compared to measurement before maternal hypoxemia (MH) experiments).

Maternal hypoxemia experiments

A total of 14 maternal hypoxemia experiments were performed on 6 fetuses. One experiment is shown in figure 6.1. The experiments were carried out from 1 hour to 51 hours after connecting the fetal lamb to the ECMO system. The time between experiments ranged from 11.5 min to 18 hours 49.5 min (median 51.5 min). Once again the periods were determined at random. The duration of the maternal hypoxemia experiments ranged from 5 min to 1 hour 16 min (median 31 min). The duration of experiments in which the incidence of fetal breathing movements (n=5, exp=8) and LV ECoG activity (n=5, exp=8) was measured was 45.0 ± 6.4 min. Maternal and fetal blood gases before and during the maternal hypoxemia experiments are shown in table 6.2. Fetal pH and $PaCO_2$ remained constant throughout the experiments; fetal PaO_2 was slightly higher (5.37 ± 0.47 kPa) than its mean value during control (3.92 ± 0.36 kPa), it then decreased to 4.18 ± 0.47 kPa in the first 5 min of the maternal hypoxemia ($p< 0.05$). This latter value is still in the normoxic fetal range (table 6.2). During the maternal hypoxemia experiments mean arterial blood pressure was 69 ± 3 mmHg and heart rate was 169 ± 9 bpm.

Nuchal EMG activity was present 85.6 ± 6.2% of the time during HV ECoG activity. Rapid eye movements (n=2) were present during LV ECoG activity and were associated with fetal breathing movements. Fetal breathing movements were only present during LV ECoG. The incidence of LV ECoG activity was 44.3 ± 7.4% and the mean length of the LV ECoG periods was 17.4 ± 2.3 min (table 6.1). Breathing activity

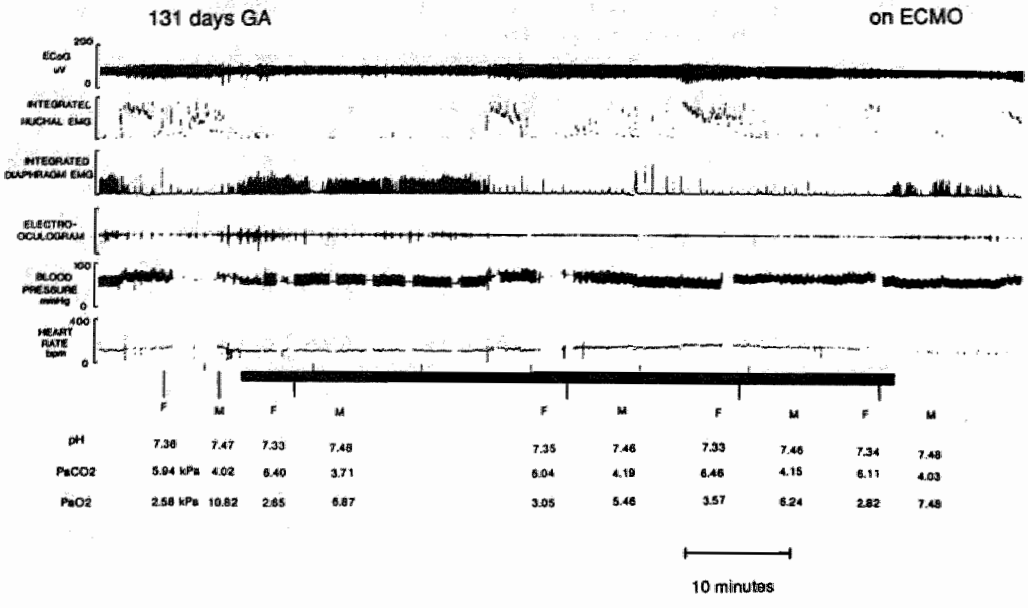

Figure 6.1 Intrauterine recording of a fetus of 131 days of gestation connected to the ECMO system 3 days after surgery. Tracings are from the top: ECoG activity, integrated nuchal EMG, integrated diaphragm EMG, electro-oculogram, blood pressure and heart rate. At the indicated times fetal (F) and maternal (M) arterial blood samples were taken for blood gases and pH. Breathing activity and rapid eye movements remain present during LV ECoG activity despite maternal hypoxemia (represented by the black bar). Nuchal EMG activity is present and associated with HV ECoG activity.

was present 21.4 ± 3.5% of the time ($p < 0.05$ compared to the incidence during baseline recordings on ECMO) and the mean length of the fetal breathing periods was 10.5 ± 1.7 min (table 6.1). Of the 14 experiments breathing activity stopped in 7 experiments within 7 min; in 7 experiments it stopped between 7-23 min after starting maternal hypoxemia. When fetal breathing stopped (within 7 min in 7 experiments) this was associated with a switch to HV ECoG and the occurrence of nuchal EMG activity. In only one experiment did fetal breathing stop in LV ECoG.

Discussion

Using an ECMO system made it possible to maintain fetal oxygenation while the maternal tissues and the maternal side of the placenta were exposed to hypoxemia. Apart from a modest decrease in the incidence of breathing activity, the well established fetal effect of acute maternal

hypoxemia, e.g. a cessation of fetal breathing movements, did not occur despite the fact that we reduced maternal PaO_2 to levels previously reported to produce cessation of fetal breathing activity. This allows us to conclude that the overall fetal response to hypoxemia previously described when using maternal hypoxemia (Boddy et al. 1974) or utero-placental hypoxemia (Bocking & Harding 1986) does not seem to be mediated by the release of substances produced by the ewe or the maternal side of the placenta.

The ECMO system itself could theoretically influence fetal behavioral activity. To control for this, we analyzed the incidence and duration of periods of fetal breathing movements and of LV ECoG activity before and after fetuses were connected to the ECMO system, while fetal blood gases and pH were held in the normal range. There was no statistical difference in the incidence or duration of periods of fetal breathing movements or LV ECoG activity before and on ECMO. Furthermore, after connection to the ECMO system, there was normal cycling of fetal states where nuchal EMG activity was associated with HV ECoG activity and rapid eye movements with LV ECoG activity. There was no statistical difference in blood pressure and heart rate before and on ECMO. Since there was no abnormal behavioral activity during the baseline periods on ECMO, it is unlikely that the use of extracorporeal circulation by itself influenced our results.

The effects of acute hypoxemia on fetal breathing activity, induced by giving the ewe a hypoxic gas mixture to breathe or by decreasing uterine blood flow, have been analyzed in different ways. Investigators have described the immediate effect of acute hypoxemia on fetal breathing movements (Blanco et al 1983b, Boddy et al. 1974, Clewlow et al. 1983, Koos et al. 1987a) and on the length of periods of fetal breathing movements (Koos et al. 1987a), or they have described the sustained effect of hypoxemia on the incidence of fetal breathing movements per hour (Boddy et al. 1974, Koos et al. 1987a). ECoG activity was also analyzed in different ways (Adamson et al. 1984, Blanco et al. 1983b, Bocking et al. 1988, Bocking & Harding 1986, Clewlow et al. 1983, Koos et al. 1987a) yielding contradictory results (Adamson et al. 1984, Bocking et al. 1988, Bocking & Harding 1986, Boddy et al. 1974, Koos et al. 1987). Combining the findings from the diverse methods it can be concluded that hypoxemia produces an acute response within 7 min which is sustained for at least one hour. Therefore we chose to analyze the immediate effects on the fetus within 5 min of induction of acute maternal hypoxemia in terms of fetal breathing activity, the length of periods of fetal breathing movements, LV ECoG activity and cardiovascular responses. We then analyzed the sustained effects of hypoxemia by describing the incidence of fetal breathing movements, LV ECoG activity, and the presence of nuchal EMG activity and rapid eye movements over the whole period of hypoxemia. In this way we felt that we would obtain a more precise interpretation of the response.

After exposing the ewe to a hypoxic gas mixture, it takes 2.5-5 min before the fetus becomes hypoxemic and shows a response (Blanco et al. 1983b, Clewlow et al. 1983, Koos et al. 1987a). Within 7 min of making

the ewe hypoxemic (maternal PaO_2 6.0 ± 0.6 kPa) we expected to see a decrease in fetal breathing activity if this was due to the release of substances from maternal tissues or the maternal side of the placenta. In our experiments fetal breathing activity stopped within 7 min in only 7 of the 14 experiments and it did not appear to be a typical response to acute hypoxemia as described previously (Clewlow et al. 1983, Koos et al. 1987a) because nuchal muscle activity and rapid eye movements were present. In the rest of the experiments fetal breathing activity stopped too late (between 7-23 min in 7 of the 14 experiments) to be the result of acute hypoxemia. If the release of substances of maternal or placental origin during hypoxemia were the mechanism responsible for fetal inhibition, this inhibition should have been manifest within 7 min. The fact that fetal breathing movements eventually stopped was to be expected, due to their periodic nature. Furthermore, in our experiments the length of periods of fetal breathing movements was not significantly shortened by exposing the ewe to hypoxemia as occurs during fetal hypoxemia (Koos et al. 1987a).

During our maternal hypoxemia experiments there was no significant evidence of a fetal cardiovascular response. Catecholamines are known to cross the placenta (Gu & Jones 1986), although the increase in fetal plasma catecholamines is thought most likely to be of fetal (Cohen et al. 1982) than of maternal (Kitterman et al. 1983) origin. It therefore appears that, as the fetus remained normoxic or mildly hyperoxic, fetal carotid chemoreceptors or the adrenal medulla were not stimulated (Blanco et al. 1984).

The incidence of fetal breathing movements is reported to decrease to levels of 0-10% when the ewe is exposed to severe hypoxemia (PaO_2 < 50 mmHg, approximately 6.6 kPa)(Adamson et al. 1984, Boddy et al. 1974, Koos et al. 1987a). In our experiments maternal PaO_2 was 6.0 ± 0.6 kPa (45 ± 5 mmHg) and the incidence of fetal breathing activity decreased significantly, but only to 21%. This may have been due to the small fall in fetal PaO_2, from mildly hyperoxic levels (5.37 ± 0.54 kPa) to the upper normoxic range (4.18 ± 0.47 kPa). However, it is not expected that this small fall in fetal PaO_2 would produce inhibition unless adaptation to the new level occurred rapidly. There is some indirect evidence which could suggest that this is the case (Matsuda et al. 1992), although the experimental conditions, the time-scale and design are very different. If the decrease in PaO_2 played a role this would again suggest that PaO_2 and not hormonal mediators were responsible for the well described response during maternal hypoxemia. Furthermore, the decrease in incidence of fetal breathing movements was not associated with a decrease in nuchal muscle activity and rapid eye movements or a change in ECoG in our experiments. Thus the mechanism normally involved in the effects of hypoxemia on the fetus (Blanco et al. 1983b, Bocking & Harding 1986, Koos et al. 1987, Natale et al. 1981, Woudstra et al. 1990) was not induced in our experiments. Our results do not support therefore the idea of delayed transfer of an inhibitor.

We are aware that our protocol would not permit us to rule out a possible role of substances produced by the *fetal* side of the placenta and

the membranes during hypoxemia because in our experiments these tissues remained normoxic or mildly hyperoxic. However, the fetal PaO_2 remained less than maternal PaO_2, thus the normal direction of O_2 transport from mother to fetus was preserved. The role of the maternal circulation in preserving placental function is underscored by the observation that the placenta and fetal membranes remain intact and functional after total fetectomy in monkeys and baboons (Albrecht et al. 1989, Albrecht & Pepe 1985, Nathanielsz et al. 1992). In addition the release of any substance from the fetal side of the placenta or membranes during hypoxemia has not been reported. Whilst fetal membranes and fetal cotyledons contain high concentrations of prostaglandins (Kelleman et al. 1992, Olson et al. 1986) and prostaglandins are known to reduce fetal breathing activity (Kitterman et al. 1983), it is reported that fetal prostaglandins do not increase during the first hour of acute hypoxemia (Akagi et al. 1990a, Akagi & Challis 1990b). Therefore, a role of prostaglandins as mediator for the inhibitory effect of breathing activity during acute hypoxemia is unlikely. During hypoxemia the placenta releases adenosine (Slegel et al. 1988) but it seems that adenosine produced in the CNS during fetal hypoxemia might have a more important role in the inhibition of fetal activity (Koos et al. 1992). Furthermore, fetal breathing movements were also inhibited when the O_2 availability was reduced by fetal methemoglobinemia, fetal carboxyhemoglobinemia or fetal anemia (Koos et al. 1988b, Koos et al. 1990a, Koos et al. 1987b). These results provide additional support for our hypothesis that the hypoxemic inhibition of fetal breathing is not initiated by the ewe or the maternal side of the placenta.

In conclusion, the well described fetal response obtained by exposing the ewe to acute hypoxemia (PaO_2 - 6kPa) was not seen when the fetus was held normoxic or mildly hyperoxic using ECMO. Therefore, the mechanism responsible for the inhibition of fetal activity during maternal hypoxemia does not seem to be mediated by the release of some inhibitory factors from the maternal side of the placenta or the ewe.

Chapter 7

Fetal breathing is not initiated after cord occlusion in the unanesthetized fetal lamb in utero

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Abstract

We investigated the role of cord occlusion in the initiation of breathing at birth using an extracorporeal membane oxygenator system to control fetal blood gases independently of the placenta in 12 chronically instrumented fetal lambs. In group IA (n=9, exp=12) $PaCO_2$ was kept constant (5.62 ± 0.21 to 5.70 ± 0.23 kPa) during cord occlusion. Group IB (n=7, exp=8) were cord occlusion experiments from group IA in which no fetal breathing movements had occurred; CO_2 flow to the membrane was increased and fetal $PaCO_2$ rose significantly (5.45 ± 0.24 to 8.27 ± 0.56 kPa). In group II (n=7, exp=12) $PaCO_2$ was allowed to increase from 5.98 ± 0.24 kPa to 8.09 ± 0.48 kPa after cord occlusion. Within 5 min of cord occlusion, fetal breathing movements did not occur in 11 out of 12 experiments in group IA or in 11 out of 12 experiments in group II. In contrast in group IB breathing did occur in 5 out of 8 experiments. When they occurred, fetal breathing movements were always associated with LV ECoG. Our results do not support the hypothesis that the initiation of breathing within 5 min of birth is dependent on an inhibitory factor of placental origin. Furthermore these data suggest an association between the presence of breathing and a substantial rise in $PaCO_2$.

Introduction

At birth pulmonary gas exchange must be established after clamping the umbilical cord. This can only happen when the lungs are expanded with air or oxygen and when there is a sufficient respiratory drive to produce and maintain respiratory efforts.

In utero, fetal breathing movements are episodic, being present only during periods when the fetus shows rapid eye movements and LV ECoG activity (Dawes et al. 1972). These fetal breathing movements are stimulated by hypercapnia (Boddy et al. 1974, Bowes et al. 1981b, Chapman et al. 1980, Dawes et al. 1982, Rigatto et al. 1988) and inhibited during acute hypoxemia (Boddy et al. 1974). In contrast, after birth breathing becomes continuous throughout all behavioural states, it is stimulated by hypoxia and is regulated by metabolic requirements. The transition from episodic fetal breathing to continuous neonatal breathing occurs at a time when many other changes occur and it is therefore difficult to isolate any single factor as being responsible for the initiation of breathing activity at birth.

The removal of the umbilical circulation has been proposed as a mechanism which initiates continuous breathing at birth. It is hypothesized (Adamson et al. 1987, Blanco et al. 1987b) that cord occlusion interrupts the supply of a factor of placental origin which prevents fetal breathing movements from becoming continuous in utero; thus cord occlusion permits the establishment of continuous breathing.

Adamson et al. (1987) and Blanco et al. (1987b) used mechanical ventilation or continuous positive pressure to oxygenate the fetus in utero after clamping the cord. Breathing was initiated after cord clamping in these experiments, and it stopped abruptly when the cord occlusion was released. However, despite the use of mechanical ventilation or continuous positive pressure in these experiments fetal $PaCO_2$ increased substantially. The reason for the initiation of continuous breathing after cord clamping in these experiments is thus not clear because the exclusion of a possible placental factor and hypercapnia occurred simultaneously. In order to clarify further the mechanism for the initiation of breathing at birth we have used an extracorporeal membrane oxygenation system to control fetal $PaCO_2$ after cord clamping. This has allowed us to address the questions a) Does the exclusion of the umbilical circulation per se play a role in the initiation of breathing at birth, and b) Does a rise in $PaCO_2$ play a role during this transition?

Materials and methods

Experiments were performed on unanesthetized chronically instrumented fetal sheep in utero (see Chapter 3).

Baseline data on incidence of fetal breathing activity and its relationship with LV ECoG were obtained before connecting the fetus to the ECMO system and after connection to the ECMO system. The effect of cord clamping on the establishment of continuous breathing was evaluated in three groups. After cord occlusion in utero there will always be an increase in fetal $PaCO_2$ since the placenta is excluded. Therefore in Group IA we decreased the CO_2 flow to the membrane in order to compensate for this and to keep fetal $PaCO_2$ constant. Group IB consisted of fetuses of group IA which did not show breathing activity after 5 min of cord occlusion; in these animals $PaCO_2$ was raised by increasing the CO_2 flow to the membrane oxygenator. In group II the CO_2 flow to the membrane oxygenator was not changed, therefore fetal $PaCO_2$ was allowed to increase after cord occlusion. Complete occlusion of the cord was confirmed in each fetus by stopping the pump of the ECMO system for about 15 seconds at the end of cord occlusion: this produced a transient bradycardia and rise in blood pressure which were reversed when the pump was restarted. Also we verified during surgery and after birth that the cord occluder could be inflated tightly around the umbilical cord and could not leak. After the experiments we clamped the cord, sacrificed the mother with an overdose of barbiturate, and delivered the fetus by caesarean section. Meanwhile the fetus was supported with the ECMO system and blood gases were kept constant. After cord occlusion 5 min were allowed for breathing to be initiaited. If it was not present after that period elapsed, we classified the experiments as negative with the respect to initiation of breathing.

Wilcoxon signed rank test was used for statistical comparison of blood gas values before and during cord occlusion and for statistical comparison of the heart rate before and after cord occlusion.

Results

Experiments were performed on 12 fetal lambs, at 131-135 days gestational age.

Six of the twelve ewes showed signs of uterine contractions on the intrauterine pressure record and were judged to be in early labour.

Baseline recordings before connecting to the extracorporeal membrane oxygenation system

The baseline recordings were obtained 55 to 70 hours after the operation. Six ewes were in labour: in those preparations fetal breathing activity was present 17.8 ± 4.8% of the time and LV ECoG activity occurred 39.3 ± 6.8% of the time. In the other six ewes which were not in labour fetal breathing activity was present 27.3 ± 4.5% of the time and LV ECoG activity occurred 51 ± 2.6% of the time. Nuchal muscle activity was always associated with HV ECoG. Fetal blood gases prior to connection to ECMO system were: pH 7.35 ± 0.03, $PaCO_2$ 6.14 ± 0.24 kPa, PaO_2 2.18 ± 0.17 kPa.

Baseline recordings after connecting to the ECMO system

On the third day post surgery the fetuses were connected to the ECMO system. In the fetuses not exposed to labour we analyzed the incidence of fetal breathing activity during periods of normal fetal blood gases (pH 7.37 ± 0.03, $PaCO_2$ 5.99 ± 0.20 kPa and PaO_2 4.20 ± 0.39 kPa). These recordings were obtained in some animals from one hour after connection to the ECMO system (n=3) and in the other three at different times between experiments before or after cord occlusion. These periods ranged from 120 to 692 min and were not meant to be used as control for the cord occlusion experiments but to control for possible deleterious effects of ECMO on normal fetal behaviour. Breathing activity was present 36.20 ± 3.75% of the time, LV ECoG activity 49.20 ± 3.37% of the time. Furthermore all 12 fetuses presented breathing movements when challenged with hypercapnia (n=9) before cord occlusion experiments, or when they were delivered at the end of the experiments (n=7). A total of 24 cord occlusion experiments were performed in the 12 fetuses.

Cord occlusion experiments

The cord occlusion experiments in all groups were carried out 91 min to 24 hours after connecting the fetal lamb to the ECMO system. The time between cord occlusion experiments varied from 28 min to 12 hours and 5 min (mean 176 ± 65 min). The effect of cord clamping on the

Table 7.1 **Fetal pH and blood gases values just before and during cord occlusion experiments in all groups.**

		pH	$PaCO_2$ (kPa)	PaO_2 (kPa)
Group IA	before	7.37 ± 0.02	5.62 ± 0.21	3.80 ± 0.27
	during	7.32 ± 0.03[1]	5.70 ± 0.23	4.19 ± 0.47
Group IB	before	7.40 ± 0.02	5.45 ± 0.24	3.86 ± 0.25
	during	7.20 ± 0.03[1]	8.27 ± 0.56[1]	6.28 ± 1.36[1]
Group II	before	7.37 ± 0.01	5.98 ± 0.24	6.27 ± 0.80
	during	7.27 ± 0.02[1]	8.09 ± 0.48[1]	5.76 ± 1.07

Means ± SEM of fetal blood gases and pH. ([1] $p \leq 0.05$, compared to values before cord occlusion).

initiation of breathing was evaluated in three groups of fetuses. Fetal blood gases on extracorporeal membrane oxygenation system before and during the cord occlusion experiments are shown in table 7.1.

When the cord was occluded there was an increase of 5 ± 1.73 mmHg in the arterial blood pressure which returned to control within 3 min. Heart rate before cord occlusion was 162 ± 6.8 bpm, and after cord occlusion it was 150 ± 8 bpm (p= 0.262). In 19 of the experiments fetal core temperature remained constant; in 3 experiments it decreased by 1.2 °C, and in 2 it increased by 0.6 °C. During cord occlusion nuchal muscle activity was associated with HV ECoG activity and not with LV ECoG activity.

Group IA

This group consisted of 9 fetuses (labour n=5) and 12 cord occlusion experiments (labour n=7). By protocol the $PaCO_2$ in this group was not allowed to increase significantly during cord occlusion (table 7.1). The duration of the cord occlusion experiments was 31 ± 5.7 min. At the moment of cord occlusion 8 fetuses were in HV ECoG activity; 3 of them switched to LV ECoG activity within 5 min. One fetus was in LV ECoG activity and switched to HV ECoG activity within 5 min. In 3 experiments ECoG could not be analyzed. In 2 experiments the cord was occluded during a period of breathing activity. This breathing activity had been present for 3 and 4.5 min respectively and it stopped 2 and 3.5 min after cord occlusion, coinciding with a swith to HV ECoG activity. These experiments were not considered positive. In group IA, fetal breathing movements did not occur in 11 of 12 experiments after cord occlusion. In one experiment breathing activity started 1 min after cord occlusion and electrocortical activity switched immediately to LV ECoG activity. Breathing activity was present during LV ECoG or intermediate electrocortical activity during this cord occlusion. This

Table 7.2 **Initiation of fetal breathing movements within 5 min after cord occlusion in utero. Also shown are the percentage changes of fetal $PaCO_2$ and the duration of the cord occlusions.**

	Group IA, n=9, exp=12	*Group IB, n=7, exp=8*	*Group II, n=7, exp=12*
FBM present (all)	1/12	5/8	1/12
FBM (labour)	1/7	4/4	0/5
FBM (non-labour)	0/5	1/4	1/7
% change in $PaCO_2$	1.4 ± 1.4	52 ± 8.4	35 ± 5.3
Duration of cord occlusions (min)	31 ± 5.7	23.6 ± 4.8	31.3 ± 3.2

Presence of fetal breathing movements within 5 min after cord occlusion in utero. Means ± SEM of the percentage changes of fetal $PaCO_2$ and the duration of cord occlusions. (FBM; fetal breathing movements, %; percentage change of $PaCO_2$, exp; experiments).

experiment was considered positive. Therefore the initiation of fetal breathing movements after cord occlusion occurred in 1 out of 12 experiments.

Group IB

This group consisted of 7 fetuses (labour n=3) and 8 of the 10 cord occlusion experiments (labour n=4) of group IA in which no breathing activity occurred. In these experiments extra CO_2 was added to the ECMO system following protocol, and the $PaCO_2$ for this group increased from 5.45 ± 0.24 kPa to 8.27 ± 0.56 (table 7.1). PaO_2 also increased from 3.86 ± 0.24 kPa to 6.28 ± 1.36 kPa (p=0.05) when the CO_2 flow to the membrane oxygenator was increased. The duration of these experiments was 23.6 ± 4.8 min. In 5 experiments the fetuses were in HV ECoG at the moment of adding CO_2 to the system. One remained in HV ECoG over the next 5 min, the other 4 switched to LV ECoG within 5 min; in 3 experiments ECoG activity could not be analyzed. Breathing activity occurred in 5 out of 8 cord occlusions (table 7.2). When present, fetal breathing movements were always associated with LV ECoG. All fetuses not exposed to labour presented fetal breathing movements during cord occlusion and hypercapnia despite that the change in $PaCO_2$ was not significantly different (labour group 52 ± 10%, non-labour group 51.85 ± 15.1%).

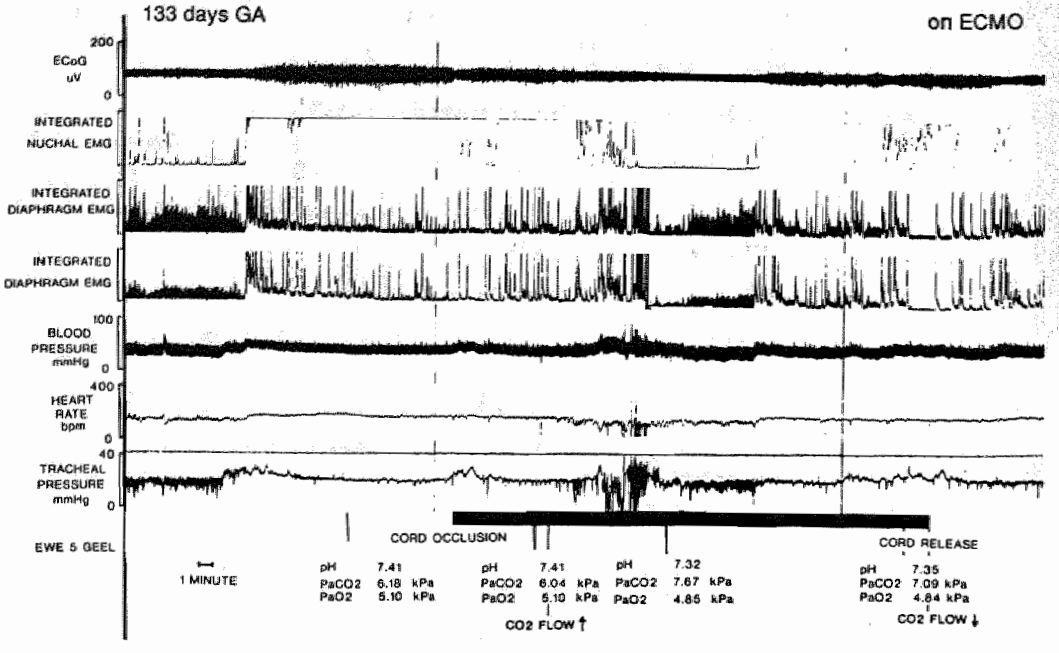

Figure 7.1 A recording from a fetus from Group IA, in utero, at 133 days gestation. Tracings are from the top: ECoG activity, integrated nuchal EMG, integrated diaphragmatic EMG (2 tracings), blood pressure, heart rate and tracheal pressure. At the indicated times the cord was occluded and released (represented by black bar), CO_2 flow to the membrane was increased or decreased, and fetal blood gases and pH samples were taken. Breathing movements were present before cord occlusion. In the first 6 min after cord occlusion PaO_2 remained constant and there was no breathing activity present. After increasing CO_2 flow to the membrane (Group IB) breathing movements were present during LV ECoG activity.

Group II

This group consisted of 7 fetuses (labour n=3) and 12 cord occlusion experiments (labour n=5). Fetal $PaCO_2$ increased from 5.98 ± 0.24 kPa to 8.09 ± 0.48 kPa during cord occlusion for this group (table 7.1). The duration of the cord occlusion experiments was 31.3 ± 3.08 min. At the moment of cord occlusion, the fetuses were in HV ECoG in 4 experiments and they remained in this state in the experiments the first 5 min of cord occlusion; in 3 experiments they switched from HV ECoG to LV ECoG within 5 min; in 2 experiments the fetuses were in LV ECoG and switched to HV ECoG within 5 min; in 2 experiments the fetuses were in LV ECoG and remained in this state for the first 5 min. In one experiment ECoG could not be analyzed. Breathing started after cord occlusion in only one experiment in this group: this was 4 min after cord occlusion and the fetus was in LV ECoG and not in labour. In one experiment the cord was occluded during a period of fetal breathing activity. Breathing was already present for 28 min, and it stopped 3.5 min

after cord occlusion. This was associated with a switch to HV ECoG activity. This experiment was considered negative.

Discussion

Our experiments were not designed to compare the incidence of fetal breathing movements before and after cord occlusion, but merely to examine whether or not cord occlusion by itself played a role in the onset of breathing. Thus we reasoned that if the exclusion of the placental circulation was the key component in the initiation of breathing at birth, then it should be initiated, after a labour period, within 60 to 90 seconds of clamping the cord (Mortola et al. 1982, Vyas et al. 1981). This should occur even if the fetus remained in utero and blood gases and core temperature were prevented from changing. Such breathing should occur regardless of the fetal ECoG state and whether the fetus was already showing fetal breathing movements or not due to the occurrence of labour. The experiments reported in this paper showed that breathing activity in utero was only established on 8% of occasions within 5 min after umbilical cord occlusion in fetal lambs supported with an ECMO system to prevent changes in fetal $PaCO_2$. This was irrespective of whether or not these ewes were in labour. When fetal breathing movements did occur, they were always associated with LV ECoG.

In order to control for the theoretically influence of an extracorporeal circuit in fetal behaviour we analyzed the incidence of fetal breathing movements during ECMO with normal fetal blood gases. We did not find a normal incidence of fetal breathing movements in our 6 animals in labour due to the inhibitory effects of labour on fetal breathing activity (Richardson et al. 1979, Kanaan et al. 1991, Molteni et al. 1980). However, we did find an normal incidence of fetal breathing activity during ECMO during periods of normal fetal blood gases in five fetuses not exposed to labour (36.20 ± 3.75%) which is similar to the incidence reported by other investigators (Boddy et al. 1974). Furthermore, in every fetus breathing could be stimulated by hypercapnia before cord occlusion or initiated postnatally in those fetuses which were delivered by caesarean section while supported by ECMO. It is therefore unlikely that the use of extracorporeal circulation by itself could have influenced our results. It is difficult and perhaps naïve to try to isolate a single factor responsible for the establishment of continuous breathing at birth because physiologically many changes happen simultaneously. However, the use of an ECMO system provides control of fetal blood gases before, during and after cord occlusions.

The exclusion of the umbilical circulation after cord occlusion has been proposed as a crucial mechanism for the initiation of breathing at birth. Both Blanco et al. (1987b) and Adamson et al. (1987) performed experiments in which the lungs were mechanically expanded with air or O_2 in utero to support the fetus after cord occlusion. They reported that breathing activity was established within 138 seconds (Blanco et al. 1987b) and 6 ± 1 min (Adamson et al. 1987), being associated with arousal (nuchal muscle activity during LV ECoG), although fetal $PaCO_2$

rose to very high levels after occluding the cord. It is interesting that breathing activity stopped within min of cord release despite the fact that fetal $PaCO_2$ was still at very high levels, suggesting involvement of a humoral factor dependent on the placental circulation. Our data showed that cord occlusion, in the absence of a substantial rise in $PaCO_2$ and in the absence of arousal, did not initiate breathing.

Recently, Adamson, Kuipers & Olson (1991) attempted to control fetal $PaCO_2$ using a high frequency ventilation system. Under these conditions the mean breathing was stimulated significantly after 20 min of umbilical cord occlusion compared to a 20 min control period, Adamson et al. (1991) suggested that this was due to a slow disappearance of a respiratory modulator after cord occlusion. Our study was designed to observe the presence of breathing within 5 min of cord clamping and not mechanisms involved in the maintenance of breathing thereafter. There are several modulators which can regulate breathing activity directly as endorphins and adenosine (Chernick 1981, Grunstein et al. 1981, Koos et al. 1990b, McQueen 1983, Santiago & Edelman 1985), prostaglandins or prostaglandin synthetase inhibitors, (Kitterman et al. 1979, Kitterman et al. 1983, Murai et al. 1987, Wallen et al. 1986) or indirectly through peripheral chemoreceptor discharge (Bennet & Hanson 1990a). Our experiments did not however support the hypothesis that abrupt changes of such modulators in fetal plasma after exclusion of the placenta were responsible for the *onset* of breathing at birth.

Initiation of breathing after cord occlusion was no more frequent in the fetuses in group II in which $PaCO_2$ was allowed to increase by ca. 34% than in group IA in which $PaCO_2$ was held at control. In group II PaO_2 was held at levels which were hyperoxic for the fetus, although not for the neonate. This might in part offset the effect on oxygen content produced by the addition of maternal blood to the fetal circulation (P_{50} adult blood higher than fetal blood). Nevertheless fetal breathing movements occurred on only one of eleven occasions. In group IB PaO_2 increased to the same level as in group II but breathing activity was observed in 5 out of 8 and 1 out of 11 experiments respectively, and fetal breathing never became continuous. These experiments support earlier studies of Blanco et al. (1991) in which raising the fetal PaO_2 to neonatal levels with ECMO failed to induce changes in the incidence of breathing; nor did such breathing occur when fetal PaO_2 was raised by expanding the lungs with mechanical ventilation (Blanco et al. 1987a).

The recent study of Baier, Hasan, Cates, Hooper, Nowaczyk & Rigatto (1990) also provided evidence that cord occlusion by itself did not induce continuous breathing. They observed continuous fetal breathing movements in chronically instrumented fetal sheep when the lungs were expanded with hyperoxic gas to elevate PaO_2 to about 200 mmHg, but not if the PaO_2 was elevated to only about 100 mmHg. If the fetuses were breathing, subsequent cord occlusion increased the intensity of this breathing; however, if they were not breathing, cord occlusion induced breathing, but this was associated with a rise in PaO_2 to about 190 mmHg. However, the unphysiologically high levels of PaO_2 used in most of these experiments raise additional questions, as such levels of

PaO_2 produce cerebral vasoconstriction (Blanco et al. 1988): a rise in the $PaCO_2$ in the environment of the medullary chemoreceptors might therefore be involved in the stimulation of breathing. However, it is clear that an elevation of $PaCO_2$ alone is not sufficient to produce continuous breathing: Baier et al. (1990) did not induce continuous breathing after clamping the cord in 5 fetuses in which PaO_2 was not elevated but $PaCO_2$ rose to about 90 mmHg, and in our experiments in which $PaCO_2$ was elevated substantially (Group IB) breathing only occurred in 5 out of 8 experiments and then only during LV ECoG.

Recently a relationship between initiation of breathing and gestational age has been reported (Hasan & Rigaux 1991). These authors could not induce fetal breathing movements in fetal lambs below 134 days of gestation by increasing fetal PaO_2 to neonatal levels. It is well known that premature infants initiate breathing as early as 22 weeks gestational age. Therefore we do not think that gestational age was a factor which impaired the initiation of breathing in our work. Furthermore, fetal PaO_2 is not expected to be above fetal levels, actually lower, with the initiation of breathing at birth. Hyperoxia can *not* play a role in the initiation of breathing at birth.

The role of hypercapnia at birth has already been suggested in a previous report (Blanco et al. 1987b) in which the time to the onset of breathing was measured under different conditions in terms of fetal blood gases. It is well established that hypercapnia produces an increase in incidence, amplitude and frequency of fetal breathing movements (Boddy et al. 1974, Bowes et al. 1981b, Chapman et al. 1980, Dawes et al. 1983) and this response is present by 0.5 of gestation in fetal lambs (Ioffe et al. 1987). It is not known whether the response to hypercapnia involves the peripheral or the central chemoreceptors, although involvement of the latter seems more likely as the response occurs in hyperoxia, which reduces the CO_2 sensitivity of the peripheral chemoreceptors (Lahiri et al. 1978). Our results (group IB) supported the idea that hypercapnia played a role in the initiation of breathing after cord occlusion. However it is clearly not the only factor involved since breathing did not become continuous but occurred only in LV ECoG.

Fetal temperature remained constant during 19 cord occlusions, in 3 experiments temperature decreased, in 2 experiments temperature increased. This is at variance with reports showing an increase in fetal temperature of 0.9 ^{0}C after cord occlusion in oxygenated (PaO_2 12.6 kPa) fetal lambs (Power et al. 1986). In our experiments we did not observe these changes. This difference in observations could be explained by the differences in fetal oxygenation (Dawes & Mott 1964, Power & Longo 1975) and by the fact that the fetuses in our study were still circulated by the ECMO system.

In summary, our data showed that breathing activity was only present in only 1 of 12 occasions within 5 min after cord occlusion when fetal $PaCO_2$ was held constant. However breathing was more often (but not always) present when fetal $PaCO_2$ increased. This finding further emphasizes that multiple mechanisms are involved in the initiation of breathing at birth. Our results do *not* allow us to support the hypothesis

that the exclusion of the umbilical circulation is responsible for the *initiation* of breathing at birth. The disappearance of such putative placental factor(s) may of course facilitate the maintenance of breathing once it has started after birth.

Chapter 8

Initiation and maintenance of continuous breathing at birth

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Submitted

Abstract

Changes in $PaCO_2$ and temperature, normally occurring at the moment of birth, may play a role in the initiation and maintenance of continuous breathing. To investigate this mechanism we instrumented 5 fetal lambs of 133-135 days of gestation for chronic recording a cord occluder and catheters for later connection to an extracorporeal membrane system. ECMO was initiated in utero at a flow rate to support the fetus after cord occlusion, then the fetuses were delivered into a warm saline bath. Breathing activity was periodically present before connection to the ECMO system and on ECMO during normocapnia/normoxia but near delivery there were no breathing movements present since all ewes were in labour. After delivering the fetuses in a warm saline bath breathing movements were periodically present. After 36-192 min breathing activity became continuously present in all animals (pH 7.20 ± 0.04, $PaCO_2$ 7.35 ± 0.16 kPa and PaO_2 12.78 ± 2.51 kPa, core temperature had fallen by a mean of 1.2 °C). Furthermore, breathing activity stopped by decreasing $PaCO_2$. Maintenance of fetal $PaCO_2$ and temperature after cord occlusion delays the establishment of continuous breathing. The level of $PaCO_2$ is important in the maintenance of breathing activity during the first few hours of life.

Introduction

Fetal breathing movements are present from early in gestation. After differentiation of electrocortical activity into LV ECoG and HV ECoG, fetal breathing movements are only present during LV ECoG. The transition from episodic fetal breathing to continuous neonatal breathing occurs at a time when many other changes occur, such as exclusion of the placenta, lung expansion with air, O_2 and CO_2 contact with the airway, a decrease in pulmonary vascular resistance and an increase in pulmonary blood flow etc. Furthermore, the newborn is exposed to new afferent input like light, sound, touch and changes in temperature. Not all of these changes have the same importance. It is difficult to isolate any single factor as being responsible for the initiation of continuous breathing activity at birth.

It is reported previously that hypercapnia stimulates fetal breathing activity and an increase in $PaCO_2$ is involved in the initiation of continuous breathing at birth (Blanco et al. 1987b). Fast peripheral cooling resulted in continuous breathing activity in utero and after birth (Harned & Ferreiro 1973, Gluckman et al. 1983). Therefore, a decrease in peripheral temperature might be involved in the initiation of continuous breathing activity at birth. Recently, it has been speculated that after cord occlusion at birth, breathing activity can be initiated due to the disappearance of respiratory inhibitors produced by the placenta (Adamson et al. 1987, Blanco et al. 1987b). However, breathing activity

was not initiated within 5 min of cord occlusion in chronicle instrumented fetal lambs when the fetus was normocapnic (Kuipers et al. 1992).

The relative importance and interaction of the mechanisms involved in the initiation and maintenance of continuous breathing at birth are not well understood. In order to clarify further the mechanism for the initiation and further maintenance of breathing at birth we have used an ECMO system in chronically instrumented fetal lambs. The aim of this work was to study the role of $PaCO_2$ and temperature on the initiation and maintenance of continuous breathing after birth.

Materials and methods

Experiments were performed on unanesthetized chronically instrumented fetal sheep in utero (see Chapter 3).

Recordings were started within 48 hours after surgery. Seventy-two hours after surgery, the fetuses were connected to the ECMO system. Baseline recordings on ECMO were started at least one hour after connection to the ECMO system. To study the initiation and maintenance of continuous breathing activity after birth the fetuses were delivered while on ECMO into a warm saline bath, using the following procedure. The pump flow was increased to approximately 350-500 ml/min, then the umbilical cord was occluded. The ewe was sacrificed by injecting 20 ml Euthesate iv. Since the umbilical circulation was interrupted the fetus was not affected. The membrane lung was supplied with a gas composed of 1.5-1.9 l/min O_2, and 0.1 l/min CO_2 at a flow rate of 2.0 l/min. Adequate O_2 delivery to the fetus was achieved in two ways. First, the O_2-flow to the membrane lung was increased to achieve a higher post-membrane saturation, secondly the pump flow was increased (to 350-500 ml/min). The abdomen of the ewe was opened, the uterus was partly visualized and opened very carefully at the place were the fetal catheters, tubing and electrodes were exteriorized. A glove filled with warm saline was placed over the fetal head until the fetal head was under water in the bath, to avoid expanding of the fetal lung with air. The fetus was exteriorized down to the umbilical cord and the cord was cut. The fetus was delivered and placed in a warm saline bath (39.5 ^{0}C), taking good care of the catheters, tubing and electrodes cables. The temperature of the bath was allowed to decrease slowly, therefore first peripheral and then central temperature decreased.

Recordings were obtained before ECMO, on ECMO during normocapnia/normoxia, before and after delivering the fetuses in a warm saline bath. The temperature of the saline bath was allowed to decrease slowly, resulting in a decrease of central temperature of the lambs. The $PaCO_2$ level was maintained constant. Blood samples were taken every 15 min, neonatal blood gases and pH were measured. After establishing continuous breathing activity neonatal $PaCO_2$ was decreased by decreasing the concentration of CO_2-flow to the membrane lung. Mild hypocapnia was defined as a fetal $PaCO_2$ 1 to 2 kPa less than baseline.

Pressure deflections representing uterine activity were seen on tracheal pressure and blood pressure channels. Labour was defined when uterine contractions occurred with a frequency $p < 0.05$ per 10 min and pressure deflections of 12-30 mmHg.

Results

Experiments were performed on 5 fetal lambs, at gestational age of 133-135 days.

Baseline recordings were obtained 55-70 hours after the operation, the duration of these recordings being 10-19 hours. During baseline recordings fetal pH and blood gases were: pH 7.34 ± 0.02, $PaCO_2$ 6.30 ± 0.22 kPa, and PaO_2 2.88 ± 0.37 kPa.

Nuchal EMG was associated with HV ECoG. Eye movements (n=4) were associated with LV ECoG and fetal breathing movements. LV ECoG activity occurred 47.6 ± 2.70% of the time and the duration of the LV ECoG periods was 15.9 ± 1.87 min. Fetal breathing activity was present for 31.7 ± 3.16% of the time, the length of periods being 8.23 ± 1.13 min. The incidence of breathing activity during LV ECoG was 65.8 ± 3.67%.

On the third day post-surgery the fetuses were connected to the ECMO system. The periods of normocapnia/normoxia were studied to control for possible effects of ECMO on fetal behaviour. Fetal pH and blood gases during these baseline recordings on the ECMO system were: pH 7.37 ± 0.01, $PaCO_2$ 6.22 ± 0.06 kPa, and PaO_2 3.57 ± 0.36 kPa.

Two ewes went into labour. There were *no* breathing movements present while these 2 fetuses remained in utero on ECMO during these hours of labour. In these 2 fetuses we could not analyze fetal breathing activity during a control period on ECMO. Nuchal EMG activity was associated with HV ECoG activity. Rapid eye movements were associated with LV ECoG activity and fetal breathing movements. The incidence of LV ECoG was 47.3 ± 3.3% of the time and the length of the LV ECoG periods was 15.6 ± 2.2 min. Breathing activity was present for 36.0 ± 4.0% of the time, the length of fetal breathing periods being 9.2 ± 1.8 min. The incidence of breathing activity during LV ECoG was 75.3 ± 3.2%.

Later, just prior to cord occlusion all fetuses were exposed to spontaneous labour, therefore there were no breathing movements present before the cord occlusion. Fetal pH and blood gases during the recordings on the ECMO system just prior to cord occlusion were: pH 7.34 ± 0.02, $PaCO_2$ 7.62 ± 0.68 kPa, and PaO_2 5.14 ± 0.65 kPa. Nuchal EMG activity remained associated with HV ECoG activity. Rapid eye movements were associated with LV ECoG activity.

After delivering the fetuses into the saline bath all neonates showed periodic breathing activity, these breathing movements being associated only with LV ECoG. Neonatal pH and blood gases during this period were: pH 7.25 ± 0.03, $PaCO_2$ 7.08 ± 0.31 kPa and PaO_2 14.96 ± 6.44 kPa. LV ECoG was present in 53.9 ± 6.1% of the time,

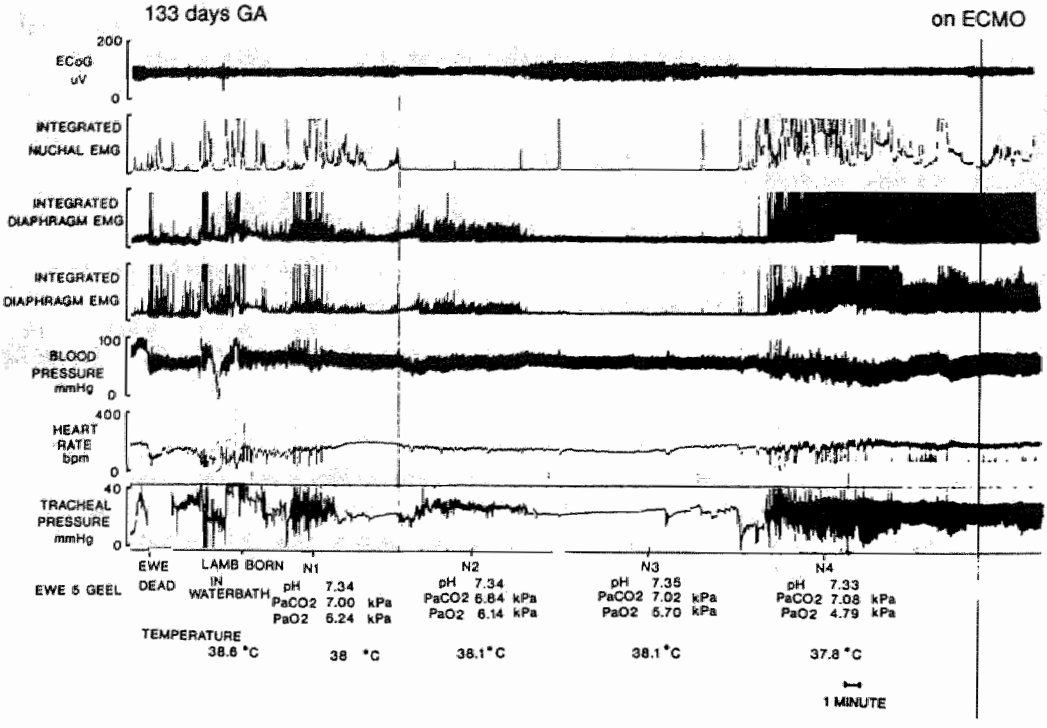

Figure 8.1 Recording of a newborn lamb at 133 days gestation of approximately 1 hour, 6 days after surgery and connected to the ECMO system. Tracings are from the top: electrocortical activity, integrated nuchal EMG, integrated diaphragm EMG, integrated diaphragm EMG, blood pressure, heart rate and tracheal pressure. Blood gas and pH samples were taken at the indicated times. Note that breathing movements were still present periodically for 36 min after delivering the newborn lamb into the saline bath. Breathing activity became present continuously after 36 min. Nuchal muscle activity was still modulated by electrocortical activity.

breathing movements were present 16.6 ± 4.0% of the total time and 30.8 ± 3.0% during LV ECoG. Nuchal EMG activity was associated with HV ECoG activity in 4 experiments, in 1 experiment nuchal EMG was also present during LV ECoG (see figure 8.1). Rapid eye movements (n=3, in one experiment electrodes were accidentally cut) were associated with LV ECoG activity and breathing movements.

After 36-192 min breathing activity became continuously present in all animals, breathing movements were continuously present both during LV ECoG and HV ECoG. At this time fetal central temperature had fallen by a mean of 1.2 °C. At the time breathing activity was initiated continuously fetal blood gases and pH were: pH 7.20 ± 0.04, $PaCO_2$ 7.35 ± 0.16 kPa and PaO_2 12.78 ± 2.51 kPa. Nuchal EMG activity was present during both LV ECoG and HV ECoG, eye movements remained associated with LV ECoG.

This continuous breathing activity was very dependent on the level of $PaCO_2$ since in all instances it could be stopped by decreasing $PaCO_2$ (pH 7.44 ± 0.02, $PaCO_2$ 3.97 ± 0.46 kPa and PaO_2 25.79 ± 6.78 kPa).

Discussion

Our results showed that breathing activity of fetuses delivered into a warm saline bath after cord occlusion and supported on ECMO was still periodic for 36-192 min. We could also show that this activity became continuous when fetal central temperature was decreased by 1.2 ^{0}C by allowing the temperature of the bath to decrease while $PaCO_2$ was maintained. Furthermore, this breathing seemed to be dependent on the level of $PaCO_2$ since it could be stopped or reversed to a periodic pattern by decreasing neonatal $PaCO_2$.

The establishment of continuous breathing at birth has been the subject of several studies. Previous investigators reported the influence of different stimuli on breathing activity at the moment of birth by means of acute experiments (Harned & Ferreiro 1973, Condorelli & Scarpelli 1975, Scarpelli et al. 1977, Moss et al. 1983). In those experiments fetal and environmental temperatures, fetal blood gases and pH were not always controlled and it is known that these factors can influence breathing activity. Therefore, the use of ECMO seemed an appropriate method to study mechanisms involved in the initiation of breathing activity since the fetus could be maintained on ECMO while fetal $PaCO_2$ and skin and core temperature could be controlled when delivered into a saline bath.

The exclusion, after cord exclusion, of respiratory modulators has been proposed as a crucial mechanism for the initiation and maintenance of breathing at birth (Adamson et al. 1987, Blanco et al. 1987b, Adamson 1991, Alvaro et al. 1993). At birth the only change in respiratory control is the presence of breathing during HV ECoG which together with the usually present breathing activity during LV ECoG results in continuous breathing. Therefore, this fetal respiratory modulator would have had only an effect on the inhibition during HV ECoG. It will have to be demonstrated that the substance responsible for periodic fetal breathing is the same modulator that disappears after cord occlusion and allows continuous breathing to happen. Moreover, we will have to distinguish or separate the mechanism involved in the initiation and those involved in the maintenance of breathing. Alvaro et al. (1993) infused placental extracts to fetuses presenting continuous breathing obtained by an unphysiologically high PaO_2. These experiments showed that the placenta contains some substances which can inhibit breathing activity. However, there are problems with the design of the experiment. First, it is not clear whether the breathing obtained is spontaneous breathing activity or just a response to unphysiologically high PaO_2 usually associated with arousal. Furthermore, it is possible that the placenta contains or produces substances which can depress or inhibit breathing activity but are not responsible for the inhibition during HV ECoG.

Our previous (Kuipers et al. 1992) and present work does not support the view that exclusion of respiratory modulators produced by the

breathing at birth for the following reasons. First, neonatal breathing was not established after cord occlusion whenever $PaCO_2$ was maintained (Kuipers et al. 1992). Secondly continuous breathing was not established until temperature decreased with $PaCO_2$ increased or maintained constant.

Breathing activity before and after birth is dependent on the level of $PaCO_2$ since we observed that in utero continuous breathing could be stopped by decreasing the level of $PaCO_2$ (Kuipers et al. 1994c). It is also now clear that the level of $PaCO_2$ plays an important role in the initiation of breathing activity at birth (Blanco et al. 1987b). For example, the initiation of continuous breathing activity is delayed during hypocapnia and stimulated during hypercapnia (Blanco et al. 1987b).

After birth there is a decrease in peripheral and central temperature of the newborn. It is known that peripheral cooling stimulates breathing activity in utero and after birth (Harned & Ferreiro 1973, Gluckman et al 1983). In our experiments breathing activity remained present periodically for 36-192 min after delivering the fetuses into a saline bath. At the time at which breathing activity became present continuously the temperature of the bath was decreased resulting in a decrease of fetal peripheral and central temperature. This indicates that a decrease in fetal temperature is an important factor involved in the initiation of continuous breathing.

So far it is well demonstrated that fetal breathing activity is dependent on the level of $PaCO_2$ but it is not very well known what the role of oxygen availability is. In utero the fetus is exposed to a low PaO_2 and since hypoxemia is inhibitory of fetal breathing activity it could be speculated that there is a continuous tonic inhibition only overridden by LV ECoG and this would be the mechanism by which fetal breathing activity is periodic. This is suggested by experiments where breathing activity became continuously present during hyperoxia (Baier et al. 1990, Hasan et al. 1991). However, these experiments were associated with an unphysiological increase of PaO_2 which is known to cause a decrease in cerebral blood flow. Furthermore, there is an increase in pulmonary blood flow bringing blood CO_2 content in contact with lung receptors sensitive for CO_2 (Blanco et al. 1988, Green & Sheldon 1983). Furthermore, in the experiments of Baier et al. (1990) and Hasan et al. (1991) there was an increase in $PaCO_2$ and a decrease in pH known stimulators of breathing activity. In chronically instrumented fetal lambs, raising fetal PaO_2 to neonatal levels (8-12 kPa) failed to induce continuous breathing activity (Blanco et al. 1987a, Blanco et al. 1991). Our results were consistent with the latter. Breathing did not become present continuously despite the increase in PaO_2 (14.96 ± 6.44 kPa) for 36-192 min. Furthermore, oxygenation cannot have a role in the initiation of continuous breathing since it increases *after* the initiation of breathing. Thus our experiments do not support the hypotheses that an increase in PaO_2 at the time of birth is involved in the initiation of continuous breathing.

It has been suggested that the ability to initiate continuous breathing is dependent on the maturity of the fetus (GA >134 days) (Hasan et al. 1991, Baier et al. 1992). In our experiments we showed that breathing activity became continuous even in fetuses of 133 days of gestation. This is lately confirmed by Tiktinsky et al. (1994).

In summary, maintenance of fetal $PaCO_2$ and temperature after cord occlusion delays the establishment of continuous breathing. The level of $PaCO_2$ is important in the maintenance of breathing activity in the first few hours of life.

Chapter 9

General discussion

Fetal breathing movements are present in utero from early in gestation. In late gestation breathing movements are present periodically. The transition from periodic fetal breathing to continuous neonatal breathing has to happen within a few minutes, and has to be effective in order to provide oxygenation. The mechanism or mechanisms involved in the control of breathing in utero are not fully understood. The presence of fetal breathing movements provides the opportunity to study the development of the control of respiration when the system is not used for survival. Furthermore, the study of the control of breathing in utero is very important since it can help to explain situations after birth like apnea in premature infants or infants with congenital hypoventilation syndrome, situations that reminds the peculiar control of breathing in utero. Any theory to explain the control of breathing in utero must consider two questions: why are fetal breathing movements present? and why are they absent during HV ECoG?

The aim of the thesis was to study mechanisms involved in the control of fetal breathing activity and in the initiation of continuous breathing at birth. Our first objective was to study the mechanism involved in determining the presence of breathing activity in utero by investigating whether the incidence of fetal breathing movements could be affected by the level of $PaCO_2$. Secondly, we investigated whether the stimulatory response of hypercapnia on fetal breathing is already present in utero and whether increased afferent input produced by cooling might change the sensitivity for CO_2 overriding the central inhibition during HV ECoG. This could shed some light on the mechanisms involved in the initiation of continuous breathing at birth. Another interesting phenomenon is the observation that fetal hypoxemia inhibits fetal breathing and behavioral activity. The mechanism responsible for this is still under discussion. We examined the possible role of maternal or placental substances released or triggered during hypoxemia which could act on the fetus. Our final objective was to study further the influence of substances produced by the placenta on the control of breathing activity. Information on the role of the placenta in controlling fetal activity is scarce. Our objective was to study whether exclusion of the placenta, and therefore the disappearance of some theoretically substances produced by it could play a role in the complicated change from periodic to continuous breathing at birth.

The control of breathing activity in utero and the initiation of continuous breathing at birth has been studied in unanesthetized chronically instrumented fetal lambs in utero over the last 20 years. This was studied in unanesthetized fetal lambs at different gestational ages by changing maternal conditions and consequently fetal conditions such as: changing maternal $PaCO_2$ (fetal hypercapnia or fetal hypocapnia), changing maternal PaO_2 (fetal hypoxemia, fetal hyperoxia), changing maternal temperature (fetal hypothermia, fetal hyperthermia), by increasing or decreasing maternal glucose levels (fetal hypoglycemia, fetal hyperglycemia), by reducing uterine blood flow (fetal hypoxemia) etc. Another approach was to change fetal conditions directly by infusing drugs, ventilating the fetal lung with different mechanical ventilator techniques such as intermittent positive pressure ventilation (IPPV),

continuous positive airway pressure (CPAP) or high frequency oscillation ventilation (HFO) etc. These methods could introduce unknown variables. For example, exposure of the ewe to hypoxemia, hypercapnia or hypocapnia, results in a change in uterine blood flow (Oakes et al. 1976, Bocking & Harding 1986, Faucher et al. 1991) or in the case of mechanical ventilation, the lung distension produces a stimulation of receptors such as stretch receptors, irritant receptors, J- receptors and also cardiovascular changes. Moreover, with these techniques it is difficult to change PaO_2 or $PaCO_2$ independently. In order to avoid some of these problems it was attractive to use a technique which approaches the fetus directly and clarify possible mechanisms in the interaction between placenta and fetus. This could be achieved by using an extracorporeal oxygen technique. ECMO systems are well developed (Cornish & Kopotic 1990) and presently used for patient care. The membrane lung presently available is capable of providing excellent gas exchange. Connection of the fetus in utero to the ECMO system allowed us to change fetal conditions independently of the ewe. For example, we had the ability to change fetal PaO_2, fetal $PaCO_2$ and temperature and to support the fetus during cord occlusion. Therefore, this technique allowed us to study the role of direct changes in O_2, CO_2 and temperature on the control of fetal breathing activity. Furthermore, we were able to design experiments to study the role of the placenta on the control of fetal breathing activity under normoxic and hypoxemic conditions.

The ECMO technique is of course invasive. It requires a sizeable (10-14 Fr) catheter in the right jugular vein and 8-10 Fr catheter in the right carotid artery. Furthermore, fetal blood is in contact with foreign surfaces such as silastic tubing, the membrane lung and it is exposed to changes in shear rate. It is therefore necessary to control for this using the same criteria as previously to judge physiological behavior, using i.e. ECoG activity, fetal breathing movements, nuchal muscle activity, rapid eye movements and cardiovascular parameters, blood gases and pH (Dawes et al. 1972, Molteni et al. 1980, Clewlow et al. 1983). It is then possible to perform experiments only on fetuses which showed comparable behavioral states, cardiovascular parameters and blood gases and pH as described in the literature. Under these conditions ECMO allowed us to control fetal blood gases, pH and central temperature. In our experiments there was no statistical difference in the incidence or duration of periods of fetal breathing movements or LV ECoG activity before ECMO and during baseline recording periods on ECMO. Furthermore, after connection to the ECMO system and maintaining fetal blood gases and pH, there was normal cycling of fetal states where nuchal EMG activity was associated with HV ECoG activity and eye movements with LV ECoG activity. There were no statistical differences in blood pressure and heart rate before and on ECMO. In conclusion, there was no abnormal behavioral activity during the baseline periods on ECMO which could influence the results presented in the thesis (chapters 4, 5, 6, 7 and 8).

Are breathing movements in utero dependent on the level of $PaCO_2$?

Whether the presence of fetal breathing activity is dependent on the level of $PaCO_2$ or it is just an expression of a sleep state is not known. But why would fetal breathing activity be dependent on $PaCO_2$? What would be the advantage of having a response to CO_2 in utero since fetal $PaCO_2$ will not be corrected by the increase in fetal breathing activity? CO_2 is the end product of aerobic metabolism and it has to be eliminated in order to maintain normal pH. Therefore it is logic that there are chemoreceptors for sensing its level and controlling the regulatory mechanisms of breathing. When these sensors are ready to react is not known but it might be speculated that early in gestation they become sensitive to CO_2 and are able to stimulate the regulatory mechanism resulting in fetal breathing activity. It is known that after birth breathing activity and $PaCO_2$ are closely related, since during hypercapnia there is an increase in breathing activity and during hypocapnia apnea occurs (Cunningham et al. 1986, Canet et al. 1993). These questions are not new since maternal hypocapnia is known to result in a decrease in presence of breathing activity of the human and the sheep fetus during the last week of gestation (Boddy et al. 1974, Connors et al. 1988, Marsál et al. 1979). However, an effect of hypoxemia could not be excluded in those experiments since there was a simultaneous decrease in uterine and umbilical blood flow (Oakes et al. 1976). Furthermore, in the human studies the experiments were limited by time and by lack of information of blood gases (Connors et al. 1988, Marsál et al. 1979). We could maintain fetal oxygenation and decrease fetal $PaCO_2$ for at least 2 hours in fetuses of 130-134 days of gestation by using ECMO. During mild hypocapnia, behavioral activity such as the incidence of LV ECoG, the length of periods of LV ECoG, the presence of eye movements during LV ECoG and the presence of nuchal EMG during HV ECoG did not change. Therefore, the possibility for the fetus to breathe remained the same, however, the incidence of fetal breathing movements decreased significantly. This shows that fetal breathing activity is not only dependent on fetal behavioral activity.

Eventhough we cannot determine since when this mechanism is present, it is clear form our experiment that the level of $PaCO_2$ is an essential determinant of the breathing activity in utero. Thus, the sensors and regulators involved in the control of breathing postnatally are already functional in utero since 130 days of gestation.

Does fetal breathing activity respond to increased levels of CO_2?

We already discussed the importance of $PaCO_2$ for the presence of fetal breathing activity. We further studied whether fetal breathing activity can be stimulated by an increased level of $PaCO_2$. It is reported that hypercapnia can stimulate breathing activity in utero (Boddy et al. 1974).

This is expressed by an increase in frequency, amplitude and incidence during LV ECoG. In those experiments fetal hypercapnia was obtained by inducing maternal hypercapnia, therefore introducing confounding variables such as changes in uterine and umbilical blood flow (Walker et al. 1976) or an increase in plasma epinephrine (Faucher et al. 1991). These results can be real since the fetus is sensitive to the level of CO_2 but we wanted to rule out some confounding factors. We obtained fetal hypercapnia by increasing the CO_2 concentration of the gas flow which supplied the membrane lung and therefore fetal $PaCO_2$ increased significantly without changing maternal conditions. Our experiments confirmed previous findings that fetal hypercapnia stimulates fetal breathing activity. The effect of such 'direct' hypercapnia was an increase in amplitude, frequency and incidence of fetal breathing movements during LV ECoG. Since there was no change in behavioral activity during hypercapnia in our experiments, there was no change in the total incidence and the length of periods of fetal breathing activity. The physiological response to CO_2 is already present in utero without any specific function. Since this mechanism is not important in utero it might be speculated that it is already present (the stimulatory effect of CO_2) in order to play an essential role in the initiation of continuous breathing at birth. Of course there are many other factors involved at the time of birth which contribute to the establishment of continuous breathing which we will discuss later.

Does the association of hypercapnia and cooling play a role in the initiation and maintenance of continuous breathing?

The mechanisms involved in the inhibition of fetal breathing activity during HV ECoG are not known. It is known that the inhibition during HV ECoG is of cortical origin and the apnea associated with it might be due to a higher threshold for CO_2 during HV ECoG (Dawes et al. 1983, Johnston & Gluckman 1989). At the time of birth the inhibition during HV ECoG must be lifted in order to breathe continuously. Since HV ECoG state continues to be present after birth its inhibitory influence must be modified. This could probably happen after birth when there is an increase in afferent input to the brain stem, the cortex and an increase in CO_2 production. At birth, there are changes in temperature which increase afferent input and simultaneously produce an increase O_2 consumption and CO_2 production. The association between cooling and an increase in CO_2 production at birth could be thought to be mechanisms involved in the initiation of continuous breathing. We could decrease fetal central temperature by decreasing the temperature of the blood in the ECMO circuit while maintaining skin temperature. In most experiments breathing activity became present continuously. In all experiments there was an increase in nuchal muscle activity during LV ECoG and HV ECoG which most probably reflected shivering.

We showed that central cooling associated with hypercapnia overrides the inhibition of breathing activity during HV ECoG resulting in

continuous breathing. It might be speculated that breathing activity could be present during HV ECoG because the extra afferent input produced by central cooling modified the inhibitory state during HV ECoG which changed the threshold for CO_2. This question is pursued further by measuring the CO_2 response during normothermia and hypothermia.

Are the inhibitory effects on fetal breathing during fetal hypoxemia an indirect effect due to release or production of mediators from the maternal side of the placenta or the ewe?

In contrast to after birth, inhibition of breathing activity is not only seen during HV ECoG but also during hypoxemia. The decrease in oxygen availability is known to inhibit fetal activity (Blanco et al. 1983b, Bocking & Harding 1986, Boddy et al. 1974, Koos et al. 1987a). The reasons for the fetal hypoxemic inhibition are not clear but there are two mechanisms proposed involving a) chemosensitive inhibitory area in the lateral pons which senses the change in PO_2 in the brain stem or b) substances produced by the ewe or the placenta during hypoxemia which directly inhibit the breathing generators. We therefore designed experiments in the attempt to separate those mechanisms. We exposed the ewe to a hypoxic gas mixture while the fetus was maintained normoxic or mildly hyperoxic on ECMO. The well described fetal hypoxemic response (Boddy et al. 1974, Blanco et al. 1983b, Clewlow et al. 1983, Bocking & Harding 1986, Koos et al. 1987a) was not seen. This could be explained as follows: If there is a chemosensitive area in the pons it was not stimulated in our experiment since PaO_2 did not fall to hypoxemic levels. We could not, however, exclude the remote possibility of a substance produced by the fetal side of the placenta and the membranes since these structures are perfused by the fetus. The design of this experiment remains a challenge.

Does the exclusion of the umbilical circulation and therefore placental modulators play a role in the initiation of breathing at birth?

At birth breathing must become continuous and for that the inhibition during HV ECoG must be overridden. It is suggested that the inhibitory state during HV ECoG could be maintained through the presence of hormones or modulators produced by the placenta. Therefore, it is very attractive to speculate that the disappearance after cord occlusion of these inhibitory agents produced by the placenta are an important factor allowing the initiation of continuous breathing activity after birth. One of the aims of our study was to examine this hypothesis. If exclusion of the umbilical circulation was the key component in the initiation of continuous breathing at birth it should be initiated within 5 min of clamping the cord regardless of the fetal blood gases, fetal ECoG state and whether or not the fetus was already showing breathing activity. In

utero after cord occlusion breathing activity did not occur within 5 min when fetal $PaCO_2$ was kept constant or increased slightly. Furthermore, breathing activity remained present periodically for 36-192 min after delivering the fetuses into a saline bath while $PaCO_2$ was held constant. Therefore, our results do not support the hypothesis that the exclusion of the umbilical circulation is responsible for the initiation of breathing at birth.

Our data suggests that not only $PaCO_2$ but changes in behavioral state, produced by an increase in afferent input such as hypothermia can stimulate breathing activity in utero and after birth (Harned & Ferreiro 1973, Gluckman et al 1983). As earlier discussed cooling associated with hypercapnia can produce continuous breathing before cord occlusion.

Moreover, it is clear that CO_2 related mechanisms are already present and important after birth. This is clearly shown in our experiments after birth where breathing activity became continuous when temperature decreased to a certain level. Whenever breathing activity became continuous, it was totally dependent on $PaCO_2$ levels since when $PaCO_2$ was reduced breathing activity stopped.

In contrast to oxygen related mechanisms which are fully developed after 24-48 hours (Blanco et al. 1984) CO_2 sensitivity seems to be already reset shortly after birth. The disappearance of placental modulators after cord occlusion did not become very clear as a crucial mechanism in our study since we could modulate the presence of breathing activity by just changing blood gases. It is not ruled out that the disappearance of these substances play a role in the maintenance of breathing activity. Further studies are being carried out to understand the mechanism.

References

Abrams RM, Gerhardt KJ, and Burchfield DJ. *Behavioral state transition and local cerebral blood flow in fetal sheep.* J. Dev. Physiol. 15: 283-288, 1991.

Adamson SL, Patrick JE, and Challis JRG. *Effects of naloxone on the breathing, electrocortical, heart rate, glucose and cortisol responses to hypoxia in the sheep fetus.* J. Dev. Physiol. 6: 495-507, 1984.

Adamson SL, Richardson BS, and Homan J. *Initiation of pulmonary gas exchange by fetal sheep in utero.* J. Appl. Physiol. 62: 989-998, 1987.

Adamson SL, Kuipers IM, and Olson DM. *Umbilical cord occlusion stimulates breathing independent of blood gases and pH.* J. Appl. Physiol. 70: 1796-1809, 1991.

Adamson SL. *Respiratory control during the transition at birth.* In: Gluckman PD, and Heymann ME Eds. Perinatal and Pediatric Pathophysiology, a clinical perspective. Edward Arnold, London, p 614-617, 1993.

Akagi K, Berdusco ETM, and Challis JRG. *Cortisol inhibits ACTH but not the AVP response to hypoxaemia in fetal lambs at days 123-128 of gestation.* J. Dev. Physiol. 14: 319-324, 1990a.

Akagi K, and Challis JRG. *Hormonal and biophysical responses to acute hypoxemia in fetal sheep at 0.7-0.8 gestation.* Can. J. Physiol. Pharmacol. Vol. 68: 1527-1532, 1990b.

Albrecht ED, Crenshaw MC, and Pepe GJ. *The effect of estrogen on placental delivery after fetectomy in baboons.* Am. J. Obstet. Gynecol. 160: 237-241, 1989.

Albrecht ED, and Pepe GJ. *The placenta remains functional following fetectomy in baboons.* Endocrinology 116: 843-845, 1985.

Alcorn D, Adamson TM, Lambert TF, Maloney JE, Ritchie BC, and Robinson PM. *Morphological effects of chronic tracheal ligation and drainage in the fetal lamb.* J. Anat. 123: 649-660, 1977.

Alcorn D, Adamson TM, Maloney JE, and Robinson PM. *Morphological effects of chronic bilateral phrenectomy or vagotomy in the fetal lamb lung.* J. Anat. 130: 683-695, 1980.

Alexander G, and Williams D. *Shivering and non-shivering thermogenesis during summit metabolism in young lambs.* J. Physiol. 198: 251-276, 1968.

Alvarez JE, Baier RJ, Fajardo CA, Nowaczyk BJ, Cates DB, and Rigatto H. *The effect of 10 % O_2 on the continuous breathing induced by O_2 or O_2 plus cord occlusion in the fetal sheep.* J. Dev. Physiol. 17: 227-232, 1992.

Alvaro R, de Almeida V, Al-Alaiyan S, Robertson M, Nowaczyk B, Cates D, and Rigatto H. *A placental extract inhibits breathing induced by umbilical cord occlusion in fetal sheep.* J. Dev. Physiol. 19: 23-28, 1993.

Andrews DC, Symonds ME, and Johnson P. *Thermoregulation and the control of breathing during non-REM sleep in the developing lamb.* J. Dev. Physiol. 16: 27-36, 1991.

Arabin B, and Riedewald S. *An attempt to quantify characteristics of behavioral states.* Am. J. Perinatol. 9: 115-119, 1992.

Bahoric A, and Chernick V. *Electrical activity of phrenic nerve and diaphragm in utero.* J. Appl. Physiol. 39: 513-518, 1975.

Baier RJ, Hasan SU, Cates DB, Hooper D, Nowaczyk B, and Rigatto H. *Effects of various concentrations of O_2 and umbilical cord occlusion on fetal breathing and behavior.* J. Appl. Physiol. 68: 1597-1604, 1990.

Baier RJ, Hasan SU, Cates DB, Hooper D, Nowaczyk B, and Rigatto H. *Hyperoxemia profoundly alters breathing activity and arouses the fetal sheep.* J. Dev. Physiol. 18: 143-150, 1992b.

Baier RJ, Fajardo C, Alvarez J, Cates DB, Nowaczyk B, and Rigatto H. *The effects of gestational age and labour on the breathing and behaviour response to oxygen and umbilical cord occlusion in the fetal sheep.* J. Dev. Physiol. 18: 93-98, 1992a.

Bamford OS, Dawes GS, Hanson MA, and Ward RA. *The effects of doxapram on breathing, heart rate and blood pressure in fetal lambs.* Respir. Physiol. 66: 387-396, 1986.

Bamford OS, and Dawes GS. *Hypoxia and electrocortical activity in the fetal lamb: effects of brainstem transection and chemoreceptor denervation.* J. Dev. Physiol. 13: 271-276, 1990.

Bamford OS, Rivera A, Tadalan T, and Ellis W. *Effects of in utero phrenic nerve section on the development of collagen and elastin in lamb lungs.* Am. Rev. Respir. Dis. 146: 1202-1205, 1992.

Banzett RB, Coleridge HM, and Coleridge JCG. I. *Pulmonary-CO_2 ventilatory reflex in dogs: effective range of CO_2 and results of vagal cooling.* Respir. Physiol. 34: 121-134, 1978.

Barcroft J. *Researches on pre-natal life.* Blackwell Scientific Publications, Oxford 1946.

Bel van F, Roman C, Iwamoto HS, and Rudolph AM. *Sympatoadrenal, metabolic, and regional blood flow responses to cold in fetal sheep.* Pediatr. Res. 34: 47-50, 1993.

Bennet L, Gluckman PD, and Johnston BM. *The central effects of thyrotrophin-releasing hormone on breathing movements and electrocortical activity of fetal sheep.* Pediatr. Res. 23: 72-75, 1988.

Bennet L, and Hanson M. *The effect of PGE2 on carotid chemoreceptor discharge in anaesthetized newborn lambs.* J. Physiol. 432: 34 P, 1990a.

Bennet L, Johnston BM, Vale WW, and Gluckman PD. *The effects of corticotrophin-releasing factor and two antagonists on breathing movements in fetal sheep.* J. Physiol. 421: 1-11, 1990b.

Berger AJ, Mitchell RA, and Severinghaus JW. *Regulation of respiration.* N. Engl. J. Med. 297: 194-201, 1977.

Berger PJ, Horne RSC, Soust M, Walker AM, and Maloney JE. *Breathing at birth and the associated blood gas and pH in the lamb.* Respir. Physiol. 82: 251-266, 1990.

Berkenbosch A, van Beek JHGM, Olievier CN, de Goede J and Quanjer PhH. *Central respiratory CO_2 sensitivity at extreme hypocapnia.* Respir. Physiol. 55: 95-102, 1984.

Bissonnette JM, and Hohimer AR. *Acute anemic hypoxemia produces a transient depression in fetal respiratory activity.* J. Appl. Physiol. 63: 1942-1946, 1987.

Bissonnette JM, Hohimer AR, Chao CR, Knopp SJ, and Notoroberto NF. *Theophylline stimulates fetal breathing movements during hypoxia.* Pediatr. Res. 28: 83-86, 1990.

Bissonnette JM, Hohimer AR, and Knopp SJ. *The effect of centrally administered adenosine on fetal breathing movements.* Resp. Physiol. 84: 273-285, 1991.

Blanco CE, Dawes GS, and Walker DW. *Effects of hypoxia on polysynaptic hind-limb reflexes in new-born lambs before and after carotid denervation.* J. Physiol. 339: 467-474, 1983a.

Blanco CE, Dawes GS, and Walker DW. *Effect of hypoxia on polysynaptic hind-limb reflexes of unanaesthetized fetal and newborn lambs.* J. Physiol. 339: 453-466, 1983b.

Blanco CE, Dawes GS, Hanson MA, and McCooke HB. *The response to hypoxia of arterial chemoreceptors in fetal sheep and new-born lambs.* J. Physiol. 351: 25-37, 1984.

Blanco CE, Martin CB Jr., Hanson MA, and McCooke HB. *Breathing activity in fetal sheep during mechanical ventilation of the lungs in utero.* Eur. J. Obstet. Gynecol. Reprod. Biol. 26: 183-192, 1987a.

Blanco CE, Martin CB, Jr., Hanson MA, and McCooke HB. *Determinants of the onset of continuous air breathing at birth.* Eur. J. Obstet. Gynecol. Reprod. Biol. 26: 175-182, 1987b.

Blanco CE, Martin CB, Rankin J, Landauer M, and Phernetton T. *Changes in fetal organ flow during intrauterine mechanical ventilation with or without oxygen.* J. Dev. Physiol. 10: 53-62, 1988.

Blanco CE, Chen V, Maertzdorf W, Bamford OS, and Hanson M. *Effect of hyperoxia (PaO_2 50-90 mmHg) on fetal breathing movements in the unanaesthetized fetal sheep.* J. Dev. Physiol. 14: 235-241, 1991.

Bocking A, Adamson L, Cousin A, Campbell K, Carmichael L, Natale R, and Patrick J. *Effects of intravenous injections on human fetal breathing movements and gross fetal body movements at 38 to 40 weeks' gestational age.* Am. J. Obstet. Gynecol. 142: 606-611, 1982.

Bocking AD, Gagnon R, Milne KM, and White SE. *Behavioral activity during prolonged hypoxemia in fetal sheep.* J. Appl. Physiol. 65: 2420-2426, 1988.

Bocking AD, and Harding R. *Effects of reduced uterine blood flow on electrocortical activity, breathing, and skeletal muscle activity in fetal sheep.* Am. J. Obstet. Gynecol. 154: 655-662, 1986.

Boddy K, Dawes GS, Fisher R, Pinter S, and Robinson JS. *Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia and hypercapnia in sheep.* J. Physiol. 243: 599-618, 1974.

Boddy K, Dawes GS, Fisher R, Pinter S, and Robinson JS. *The effects of pentobarbitone and pethidine on foetal breathing movements in sheep.* Br. J. Pharmac. 57: 311-317, 1976.

Bowes G, Adamson TM, Ritchie BC, Dowling M, Wilkinson MH, and Maloney JE. *Development patterns of respiratory activity in unanesthetized fetal sheep in utero.* J. Appl. Physiol. 50: 693-700, 1981a.

Bowes G, Wilkinson MH, Dowling M, Ritchie BC, Brodecky V, and Maloney JE. *Hypercapnic stimulation of respiratory activity in unanesthetized fetal sheep in utero.* J. Appl. Physiol. 50: 701-708, 1981b.

Brown ER, Lawson EE, Jansen A, Chernick V, and Taeusch HW. *Regular fetal breathing induced by pilocarpine infusion in the near-term fetal lamb.* J. Appl. Physiol. 50: 1348-1352, 1981.

Bryan AC, Bowes G, and Maloney JE. *Control of breathing in the fetus and the newborn.* In: Fishman AP, Cherniack NS, Widdicombe JG Eds. Handbook of Physiology section 3: The Respiratory System, Volume II, part 2. American Physiological Society, Bethesda, U.S.A., 1986, p 621-647.

Canet E, Praud J-P, Laberge J-M, Blanchard PW, and Bureau MA. *Apnea threshold and breathing rhythmicity in newborn lambs.* J. Appl. Physiol. 74: 3013-3019, 1993.

Chapman RLK, Dawes GS, Rurak DW, and Wilds PL. *Breathing movements in fetal lambs and the effect of hypercapnia.* J. Physiol. 302, 19-29, 1980.

Chernick V. *Endorphins and ventilatory control.* New Engl. J. Med. 304: 1227-1228, 1981.

Clapp III JF, Szeto HH, Abrams R, Larrow R, and Mann LI. *Physiologic variability and fetal electrocortical activity.* Am. J. Obstet. Gynecol. 136: 1045-1050, 1980.

Clewlow F, Dawes GS, Johnston BM, and Walker DW. *Changes in breathing, electrocortical and muscle activity in unanaesthetized fetal lambs with age.* J. Physiol. 341: 463-476, 1983.

Cohen WR, Piasecki GJ, and Jackson BT. *Plasma catecholamines during hypoxemia in fetal lamb.* Am. J. Physiol. 243: R520-R525, 1982.

Cohn HE, Sacks EJ, Heymann MA, and Rudolph AM. *Cardiovascular responses to hypoxemia and acidemia in fetal lambs.* Am. J. Obstet. Gynecol. 120: 817-824, 1974.

Coles SK, Kumar P, and Noble R. *Pontine sites inhibiting breathing in anaesthetized neonatal lambs.* J. Physiol. 409:66P, 1989.

Condorelli S, and Scarpelli EM. *Somatic-respiratory reflex and the onset of regular breathing movements in the fetal lambs in utero.* Pediat. Res. 9: 879-884, 1975.

Connors G, Hunse C, Carmichael L, Natale R, and Richardson B. *The role of carbon dioxide in the generation of human fetal breathing movements.* Am. J. Obstet. Gynecol. 158: 322-327, 1988.

Connors G, Hunse C, Carmichael L, Natale R, and Richardson B. *Control of fetal breathing in the human fetus between 24 and 34 weeks' gestation.* Am. J. Obstet. Gynecol. 160: 932-938, 1989.

Cooke IRC, and Berger PH. *Precursor of respiratory pattern in the early gestation mammalian fetus.* Brain Res. 522: 333-336, 1990.

Cornish JD, and Kopotic RJ. *ECMO specialist trainig manual.* The Egleston Children's Hospital ECMO-centre, Atlanta Georgia USA, 1990.

Cunningham DJC, Robbins PA, and Wolff CB. *Integration of respiratory responses to changes in alveolar partial pressures of CO_2 and O_2 and in arterial pH.* In: Fishman AP, Cherniack NS, Widdicombe JG Eds. Handbook of Physiology section 3: The Respiratory System, Volume II, part 2. American Physiological Society, Bethesda, 1986, p 475-528.

Datta AK, Shea SA, Horner RL, and Guz A. *The influence of induced hypoccapnia and sleep on the endogenous respiratory rhythm in humans.* J. Physiol. 440: 17-33, 1991.

Dawes GS, Fox HE, Leduc BM, Liggins GC, and Richards RT. *Respiratory movements and rapid eye movement sleep in the foetal lamb.* J. Physiol. 220: 119-143, 1972.

Dawes GS. *Breathing and rapid eye movement sleep before birth.* In: Comline R.S., K.W. Cross, P.W. Nathanielsz Eds. Foetal and Neonatal Physiology, Proceedings of the Sir Joseph Barcroft Centenary Symposium, Cambridge University Press, London, 1973.

Dawes GS, Gardener WN, Johnston BM, and Walker DW. *Activity of intercostal muscles in relation to breathing movements, electrocortical activity, and gestational age in fetal lambs.* J. Physiol. 307: 47-48P, 1980.

Dawes GS, Gardner WN, Johnston BM, and Walker DW. *Effects of hypercapnia on tracheal pressure, diaphragm and intercostal electromyograms in unanaesthetized fetal lambs.* J. Physiol. 326: 461-474, 1982.

Dawes GS, Gardner WN, Johnston BM, and Walker DW. *Breathing in fetal lambs: the effect of brain stem section.* J. Physiol. 335: 535-553, 1983.

Dejours P. *Chemoreflexes in breathing.* Physiol. Rev. 42: 335-358, 1962.

Dickson KA, and Harding R. *Fetal breathing and pressures in the trachea and amniotic sac during oligohydramnios in sheep.* J. Appl. Physiol. 70: 293-299, 1991.

Eldridge FL, and Millhorn DE. *Oscillation, gating, and memory in the respiratory control system.* In: Fishman AP, Cherniack NS, Widdicombe JG Eds. Handbook of Physiology section 3: The Respiratory System, Volume II, part 1. American Physiological Society, Bethesda, U.S.A., 1986, p 69-91.

Faber JJ, Anderson DF, Morton MJ, Parkes CM, Pinson CW, Thronburg KL, and Willis DM. *Birth, its physiology, and the problems it creates.* In: C.T. Jones and P.W. Nathanielsz Eds. The Physiological Development of the Fetus and Newborn, Academic press, London, U.K., 1985, 371-380.

Faucher DJ, Laptook AR, Porter JC, and Rosenfeld CR. *Effects of acute hypercapnia on maternal and fetal vasopressin and catecholamine release.* Pediatr. Res. 30: 368-374, 1991.

Fencl V. *Handbook of Physiology. Section 3: the Respiratory System Volume II, Control of Breathing, part 1.* Bethesda, American Physiological Society, 1986, p 115-140.

Ferreira SH, and Vane JR. *Prostaglandins: Their disappearance from and release into the circulation.* Nature 216: 868-873, 1967.

Fewell JE, Lee CC, and Kitterman JA. *Effects of phrenic nerve section on the respiratory system of fetal lambs.* J. Appl. Physiol. 51: 293-297, 1981.

Fisk NM, Parkes MJ, Moore PJ, Haidar A, Wigglesworth J, and Hanson MA. *Fetal breathing during chronic lung liquid loss leading to pulmonary hypoplasia.* Early Hum. Dev. 27: 53-63, 1991.

Fleming PJ, Bryan AC, and Bryan MH. *Functional immaturiy of pulmonary irritant receptors and apnea in newborn preterm infants.* Pediatr. 61: 515-518, 1978.

Fletcher DJ, Hanson MA, Moore PJ, and MJ Parkes. *Stimulation of the sheep fetus in utero by sound.* J. Physiol. 409: 42 P, 1989.

Gluckman PD, Gunn TR, and Johnston BM. *The effect of cooling on breathing and shivering in unanesthetized fetal lambs in utero.* J. Physiol. 343: 495-506, 1983.

Gluckman PD, and Johnston BM. *Lesions in the upper lateral pons abolish the hypoxic depression of breathing in unanaesthetized fetal lambs in utero.* J. Physiol. 382: 373-383, 1987.

Grunstein MM, Hazinsky TA, and Schlueter MA. *Respiratory control during hypoxia in newborn rabbits: implied action of endorphins.* J. Appl. Physiol. 51: 122-130, 1981.

Gu W, and Jones CT. *The effect of elevation of maternal plasma catecholamines on the fetus and placenta of the pregnant sheep.* J. Dev. Physiol. 8: 173-186, 1986.

Guerra FA, Savich RD, Wallen LD, Lee CH, Clyman RI, Mauray FE, and Kitterman JA. *Prostaglandins E_2 causes hypoventilation and apnea in newborn lambs.* J. Appl. Physiol. 64: 2160-2166, 1988.

Guerra FA, Savich RD, Clyman RI, and Kitterman JA. *Meclofenamate increases ventilation in lambs.* J. Dev. Physiol. 11: 1-6, 1989.

Guissani DA, Moore PJ, Bennet L, Spencer JAD, and Hanson MA. *Phentolamine increases the incidence of fetal breathing movements both in normoxia and in hypoxia in term fetal sheep.* J. Phyiol. 425: 320P, 1992.

Gunn TR, Johnston BM, Iwamoto HS, Fraser M, Nicholls MG, and Gluckman PD. *Haemodynamic and catecholamine responses to hypothermia in the fetal sheep in utero.* J. Dev. Physiol. 7: 241-249, 1985.

Haddad GG, and Mellins RB. *The role of airway receptors in the control of respiration in infants: a review.* J. Pediatr. 91: 281-286, 1977.

Hallak M, Moise KJ, Lira N, Dorman F, O'Brien Smith E, and Cotton DB. *The effect of tocolytic agents indomethacin and terbutaline on fetal breathing and body movements: A prospective, randomized, double-blind, placebo-controlled clinical trial.* Am. J. Obstet. Gynecol. 167: 1059-1063, 1992.

Hansen NB, Brubakk AM, Bratlid D, Oh W, and Stonestreet BS. *The effects of variations in $PaCO_2$ on brain blood flow and cardiac output in the newborn piglet.* Pediatr. Res. 18: 1132-1136, 1984.

Hanson MA, Moore PJ, Nijhuis JG, and Parkes MJ. *Effects of pilocarpine on breathing movements in normal, chemodenervated and brain-stem transected fetal sheep.* J. Physiol. 400: 415-424, 1988.

Harding R, Rawson JA, Griffiths PA, and Thornburn GD. *The influence of acute hypoxia and sleep states on the electrical activity of the cerebellum in the sheep fetus.* Electroencephalogr. Clin. Neurophysiol. 57: 166-173, 1984.

Harding R, Hooper SB, and Han VKM. *Abolition of fetal btreathing movements by spinal cord transection leads to reductions in fetal lung liquid volume, lung growth, and IGF-II gene expression.* Pediatr. Res. 34: 148-153, 1993.

Harned HS, and Ferreiro J. *Initiation of breathing by cold stimulation: Effects of change in ambient temperature on respiratory activity of the full-term fetal lamb.* J. Pediatr. 83: 663-669, 1973.

Hasan SU, Bamford OS, Hawkins RL, Gibson DA, Nowaczyk BJ, Cates DB, and Rigatto H. *The effects of brain-stem section on the breathing and behavioural response to morphine in the fetal sheep.* J. Dev. Physiol. 13: 147-155, 1990.

Hasan SU, Lee DS, Gibson DA, Nowaczyk BJ, Cates DB, Sitar DS, Pinsky C, and Rigatto H. *Effect of morphine on breathing and behavior in fetal sheep.* J. Appl. Physiol. 64: 2058-2065, 1988.

Hasan SU, and Rigaux A. *The effects of lung distension, oxygenation, and gestational age on fetal behavior and breathing movements in sheep.* Pediatr. Res. 30: 193-201, 1991.

Hasan SU, and Rigaux A. *Arterial oxygen threshold range for the onset of arousal and breathing in fetal sheep.* Pediatr. Res. 32: 342-349, 1992.

Hedner T, Hedner J, Wessberg P, and Jonason J. *Regulation of breathing in the rat: indications for a role of central adenosine mechanisms.* Neuroscience Letters 33: 147-151, 1982.

Hinman DJ, and Szeto HH. *Cholinergic influences on sleep-wake patterns and breathing movements in the fetus.* J. Pharmacol. Exp. Ther. 247: 372-378, 1988.

Hohimer AR, and Bissonnette JM. *Effect of metabolic acidosis on fetal breathing movements in utero.* Respir. Physiol. 43: 99-106, 1981.

Hohimer AR, and Bissonnette JM. *Vascular lactic acid infusions do not alter the incidence of fetal breathing movements or their inhibition by acute hypoxemia.* Pediatr. Res. 29: 483-486, 1991.

Hohimer AR, Bissonnette JM, Richardson BS, and Machida CM. *Central chemical regulation of breathing movements in fetal lambs.* Respir. Physiol. 52; 99-111, 1983.

Hohimer AR, Richardson BS, Bissonnette JM, and Machida CM. *The effect of indomethacin on breathing movements and cerebral blood flow and metabolism in the fetal sheep.* J. Dev. Physiol. 7: 217-228, 1985.

Ioffe S, Jansen AH, and Chernick V. *Maturation of spontaneous fetal diaphragmatic activity and fetal response to hypercapnia and hypoxemia.* J. Appl. Physiol. 62: 609-622, 1987.

Ioffe S, Jansen AH, and Chernick V. *Analysis of respiratory neuronal activity in fetal sheep.* J. Appl. Physiol. 73: 1972-1981, 1992.

Ioffe S, Jansen AH, Russell BJ, and Chernick V. *Sleep, wakefulness and the monosynaptic reflex in fetal and newborn lambs.* Plügers Arch. 388: 149-157, 1980.

Iwamoto HS, Teitel D, and Rudolph AM. *Effects of birth-related events on blood flow distribution.* Pediatr. Res. 22: 634-640, 1987.

Jansen AH, de Boeck C, Ioffe S, and Chernick V. *Indomethacin-induced fetal breathing: mechanism and site of action.* J. Appl. Physiol. 57: 360-365, 1984

Jansen AH, Ioffe S, and Chernick V. *Drug-induced changes in fetal breathing and sleepstate.* Can. J. Physiol. Pharmacol. 61: 315-324, 1983.

Jansen AH, Ioffe S, and Chernick V. *Stimulation of fetal breathing activity by beta-adrenergic mechanisms.* J. Appl. Physiol. 60: 1938-1945, 1986.

Jansen AH, Ioffe S, Russell BJ, and Chernick V. *Effect of carotid chemoreceptor denervation on breathing in utero and after birth.* J. Appl. Physiol. 51: 630-633, 1981.

Jansen AH, Ioffe S, Russell BJ, and Chernick V. *Influence of sleep state on the response to hypercapnia in fetal lambs.* Respir. Physiol. 48: 125-142, 1982.

Johnston BM, and Gluckman PD. *GABA-mediated inhibition of breathing in the late gestation sheep fetus.* J. Dev. Physiol. 5: 353-360, 1983.

Johnston BM, and Gluckman PD. *Lateral pontine lesions effect central chemosensitivity in unanesthetized fetal lambs.* J. Appl. Physiol. 67: 1113-1118, 1989.

Jones SA, Adamson SL, Bishai I, Lees J, Engelberts D, and Coceani F. *Eicosanoids in third ventricle cerebrospinal fluid of fetal and newborn sheep.* Am. J. Physiol. 264: R135-R142, 1993.

Joseph SA, and Walker DW. *Catecholamine neurons in fetal brain: effects on breathing movements and electrocorticogram.* J. Appl. Physiol. 69: 1903-1911, 1990.

Kanaan CM, O'Grady JP, and Veille JC. *Effect of maternal carbondioxide inhalation on human fetal breathing movements in term and preterm labour.* Obstet. Gynecol. 78: 9-13, 1991.

Kelleman A, Binienda Z, Ding X-Y, Rittenhouse L, Mitchell M, and Nathanielsz PW. *Prostaglandin production in the umbilical and uterine circulations in pregnant sheep at 129-136 days gestation.* J. Dev. Physiol. 17: 63-67, 1992.

Khazin AF, Hon EH, and Hehre FW. *Effects of maternal hyperoxia on the fetus.* Am. J. Obstet. Gynec. 109: 628-637, 1971.

Kitanaka T, Gilbert RD, and Longo LD. *Maternal responses to long-term hypoxemia in sheep.* Am. J. Physiol. 256: R1340-R1347, 1989.

Kitterman JA, Liggins GC, Clements JA, and Tooley WH. *Stimulation of breathing movements in fetal sheep by inhibitors of prostaglandin synthesis.* J. Dev. Physiol. 1: 453-466, 1979.

Kitterman JA, Liggins GC, Fewell JE, and Tooley WH. *Inhibition of breathing movements in fetal sheep by prostaglandins.* J. Appl. Physiol. 54: 687-692, 1983.

Kolobow T, Gattinoni L, Tomlinson TA, Pierce JE. *Control of breathing using an extracorporeal membrane lung.* Anesthesiology 46: 138-141, 1977.

Koos BJ. *Central stimulation of breathing movements in fetal lambs by prostaglandin synthetase inhibitors.* J. Physiol. 362: 455-466, 1985.

Koos BJ, Sameshima H, and Power GG. *Fetal breathing movement, sleep state and cardiovascular responses to an inhibitor of mitochondrial ATPase in sheep.* J. Dev. Physiol. 8: 67-75, 1986.

Koos BJ, Sameshima H, and Power GG. *Fetal breathing, sleep state, and cardiovascular responses to graded hypoxia in sheep.* J. Appl. Physiol. 62: 1033-1039, 1987a.

Koos BJ, Sameshima H, and Power G. *Fetal breathing, sleep state, and cardiovascular responses to graded anemia in sheep.* J. Appl. Physiol. 63: 1463-1468, 1987b.

Koos BJ, Kitanaka T, Matsuda K, Gilbert RD, and Longo LD. *Fetal breathing adaptation to prolonged hypoxaemia in sheep.* J. Dev. Physiol. 10: 161-166, 1988a.

Koos BJ, Matsuda K, and Power GG. *Fetal breathing and sleep state responses to graded carboxyhemoglobinemia in sheep.* J. Appl. Physiol. 65: 2118-2123, 1988b.

Koos BJ, and Sameshima H. *Effects of hypoxaemia and hypercapnia on breathing movements and sleep state in sinoaortic-denervated fetal sheep.* J. Dev. Physiol. 10: 131-144, 1988c.

Koos BJ, Matsuda K, and Power GG. *Fetal breathing and cardiovascular responses to graded methemoglobinemia in sheep.* J. Appl. Physiol. 69: 136-140, 1990a.

Koos BJ, and Matsuda K. *Fetal breathing, sleep state, and cardiovascular responses to adenosine in sheep.* J. Appl. Physiol. 68: 489-495, 1990b.

Koos BJ, and Doany W. *Role of plasma adenosine in breathing responses to hypoxia in fetal sheep.* J. Dev. Physiol. 16: 81-85, 1991.

Koos BJ, Chao A, and Daony W. *Adenosine stimulates breathing in fetal sheep with brain stem section.* J. Appl. Physiol. 72: 94-99, 1992.

Kuipers IM , Maertzdorf WJ, de Jong DS, Hanson MA, Blanco CE. *The effect of mild hypocapnia on breathing and behavior in unanesthetized normoxic fetal lambs.* J. Appl. Physiol. 76(4): 1476-1480, 1994a.

Kuipers IM , Maertzdorf WJ, de Jong DS, Hanson MA, Blanco CE. *Initiation and maintenance of continuous breathing at birth.* Submitted 1995b.

Kuipers IM , Maertzdorf WJ, de Jong DS, Hanson MA, Blanco CE. *The effect of hypercapnia and hypercapnia associated with central cooling on behavior in unanesthetized fetal lambs.* Submidded 1995a.

Kuipers IM , Maertzdorf WJ, de Jong DS, Hanson MA, Blanco CE. *The effect of central cooling on behavior in unesthetied fetal lambs; the role of CO_2.* Ped. Res. 35: 4; abstract 408, 1994c.

Kuipers IM , Maertzdorf WJ, Keunen H, de Jong DS, Hanson MA, Blanco CE. *Fetal breathing is not initiated after cord occlusion in the unanesthetized fetal lamb in utero.* J. Dev. Physiol. 17: 233-240, 1992.

Kuipers IM , Maertzdorf WJ, Keunen H, de Jong DS, Hanson MA, Blanco CE. *The effect of maternal hypoxemia on behavior in unanesthetized normoxic or mildly hyperoxic fetal lambs.* J. Appl. Physiol. 76: 2535-2540, 1994.

Kuwamura T, Gilbert RD, and Power GG. *Effect of cooling and heating on the regional distribution of blood flow in fetal sheep.* J. Dev. Physiol. 8: 11-21, 1986.

Lagercrantz H, and Bistoletti P. *Catecholamine release in the newborn infant.* Pediatr. Res. 11: 889-893, 1973.

Lagercrantz H, Pequignot J, Pequignot J-M and Peyrin L. *The first breaths of air stimulate noradrenaline turnover in the brain of the newborn rat.* Acta Physiol. Scand. 144: 433-438, 1992.

Lahiri S, Mokashi A, Delaney RG, and Fishman AP. *Arterial P_{O2} and P_{CO2} stimulus threshold for carotid chemoreceptors and breathing.* Respir. Physiol. 34: 359-375, 1978.

Leduc B. *The effect of hyperventilation on maternal placental blood flow in pregnant rabbits.* J. Physiol. 225: 339-348 1972.

Lee DS, Choy P, Davi M, Caces R, Gibson D, Hasan SU, Cates D, and Rigatto H. *Decrease in prostaglandin E2 is not essential for the establishment of continuous breathing at birth in sheep.* J. Dev. Physiol. 12: 145-151, 1989.

Levinson G, Shnider SM, deLorimier AA, and Steffenson JL. *Effects of maternal hyperventilation on uterine blood flow and fetal oxygenation and acid-base status.* Anesthesiology 40: 340-347, 1974.

Lewis AB, Evans WN, and Sischo W. *Plasma catecholamine responses to hypoxemia in fetal lambs.* Biol. Neonate 41: 115-122, 1982.

Liggins GG, Vilos GA, Campos GA, Kitterman JA, and Lee CH. *The effect of spinal cord transection on lung development in fetal sheep.* J. Dev. Physiol. 3: 267-274, 1981a.

Liggins GG, Vilos GA, Campos GA, Kitterman JA, and Lee CH. *The effect of bilateral thoracoplasty on lung development in fetal sheep.* J. Dev. Physiol. 3: 275-282, 1981b.

Malcolm GA, and Henderson-Smart DJ. *The effect of body temperature on the ventilatory response to CO2 in neonatal rats.* Twenty first Annual Meeting of the Society for the Study of Fetal Physiology. Palm Cove, Cairns, Australia, July 30-August 3, 1994. Abstract 58.

Marsál K, Gennser G, Löfgren O. *Effects on fetal breathing movements of maternal challenges.* Acta Obstet. Gynecol. Scand. 58: 335-342, 1979.

Marsland DW, Callahan B, and Shannon DC. *The afferent vagus and regulation of breathing in response to inhaled CO_2 in awake newborn lambs.* Biol. Neonate 27: 102-107, 1975.

Martin CB, Jr., Voermans TMG, and Jongsma HW. *Effect of reducing uteroplacental blood flow on movements and on electrocortical activity of fetal sheep.* Gynecol. Obstet. Invest. 23: 34-39, 1987.

Matsuda K, Ducsay C, and Koos BJ. *Fetal breathing, sleep state and cardiovascular adaptations to anaemia in sheep.* J. Physiol. 445: 713-723, 1992.

McCown TJ, Hedner JA, Towle AC, Breese GR, and Mueller RA. *Brainstem localization of thyrotropin-releasing hormone-induced change in respiration function.* Brain Res. 373: 189-196, 1986.

McQueen DS. *Opoid peptide interactions with respiratory and circulatory systems.* Br. Med. Bull. 39: 77-82, 1983.

Molteni RA, Melmed MH, Sheldon RE, Jones MD, and Meschia G. *Induction of fetal breathing by metabolic acidemia and its effect on blood flow to the respiratory muscles.* Am J. Obstet. Gynecol. 136: 609- 620, 1980.

Moore PJ, and Hanson MA. *Control of breathing; central influences.* Edited by MA Hanson JAD Spencer, CA Rodeck and D Walters. Fetus and neonate Physiology and clinical applications, Volume 2 Breathing. Press Syndicate Cambridge UK, 109-136, 1994.

Moore PJ, Parkes MJ, Nijhuis JG, and Hanson MA. *The incidence of breathing movements of fetal sheep in normoxia and hypoxia after peripheral chemodenervation and brain-stem transection.* J. Dev. Physiol. 11: 147-151, 1989.

Moore PJ, Parkes MJ, Noble R, and Hanson MA. *Reversible blockade of the secondary fall of ventilation during hypoxia in anaesthetized newborn sheep by focal cooling in the brain stem.* J. Physiol. 438: 242 P, 1991.

Mortola JP, Fisher JT, Smith JB, Fox JS, Weeks S, and Willis D. *Onset of respiration in infants delivered by cesarean section.* J. Appl. Physiol. 52: 716-724, 1982.

Moss IR, and Scarpelli EM. *Generation and regulation of breathing in utero: fetal CO_2 response test.* J. Appl. Physiol. 47: 527-531, 1979

Moss IR, Mautone AJ, and Scarpelli EM. *Effect of temperature on regulation of breathing and sleep/wake state in fetal lambs.* J. Appl. Physiol. 54: 536-543, 1983.

Motoyama EK, Rivard G, Acheson F, and Cook CD. *The effect of changes in maternal pH and PCO_2 on the PO_2 of fetal lambs.* Anesthesiology 28: 891-903, 1967.

Mulder EJH, Boersma M, Meeuse M, Wal van der M, Weerd van de E, Visser GHA. *Patterns of breathing movements in the near-term human fetus: relationship to behavioural states.* Early Hum. Dev. 36: 127-135, 1994.

Murai DT, Clymann RI, Mauray FE, Lee C-CH, and Kitterman JA. *Meclofenamate and prostaglandin E_2 affect breathing movements independently of glucose concentrations in fetal sheep.* Am. J. Obstet. Gynecol. 150: 758-764, 1984.

Murai DT, Lee CH, Wallen LD, and Kitterman JA. *Denervation of peripheral chemoreceptors decreases breathing movements in fetal sheep.* J. Appl. Physiol. 59: 575-579, 1985.

Murai DT, Wallen LD, Chu-Ching HL, Clyman RI, Mauray RI, and Kitterman JA. *Effects of prostaglandins on fetal breathing do not involve peripheral chemoreceptors.* J. Appl. Physiol. 62: 271-277, 1987.

Murata Y, Martin CB, Miyake K, Socol M, and Druzin M. *Effect of catecholamine on fetal breathing activity in rhesus monkey.* Am. J. Obstet. Gynecol. 139: 942-947, 1981.

Natale R, Clewlow F, and Dawes GS. *Measurement of forelimb movements in the lamb in utero.* Am. J. Obstet. Gynec. 140: 545-551, 1981.

Natale R, Patrick J, and Richardson B. *Effects of human maternal venous plasma glucose concentrations on fetal breathing movements.* Am. J. Obstet. Gynecol. 132: 36-41, 1978.

Nathanielsz PW, Figueroa JP, and Honnebier MBOM. *In the rhesus monkey placental retention after fetectomy at 121 to 130 days' gestation outlasts the normal duration of pregnancy.* Am. J. Obstet. Gynecol. 166: 1529-1535, 1992.

Nijhuis JG, Prechtl HFR, Martin CB, jr., and Bots RSGM. *Are there behavioral states in the human fetus.* Early Hum. Dev. 6: 177-195, 1982.

Noble R, and Smith JA. *Inhibition of breathing in neonatal kittens by pontine electrical stimulation.* Society for the Study of Fetal Physiology. 18th International Meeting, May 7-10, 1991, Abstract 47.

Noble R, and Williams BA. *Excitation of neurones in the rostral lateral pons during hypoxia in anaesthetized neonatal lambs.* J. Physiol. 417:146P, 1989.

O'Grady JP, Richardson B, Hohimer AR, and Burry KA. *The effect of induced maternal hypercapnia on gross fetal body movements.* Am. J. Obstet. Gynecol. 146: 52-56, 1983.

Oakes GK, Walker AM, Ehrenkranz RA, Cefalo RC, and Chez RA. *Uteroplacental blood flow during hyperthermia with and without respiratory alkalosis.* J. Appl. Physiol. 41: 197-201, 1976.

Okai T, Kozuma S, Shinozuka N, Kuwabara Y, and Mizuno M. *A study on the development of sleep-wakefulness cycle in the human fetus.* Early Hum. Dev. 29:391-396, 1992.

Olson DM, Lye SJ, and Challis JRG. *Prostaglandin concentrations in ovine maternal and fetal tissues at late gestation.* Pediatr. Res. 20: 83-86, 1986.

Pappenheimer JR, Fencl V, Heisey R, and Held D. *Role of cerebral fluids in control of respiration as studied in unanesthetized goats.* Am. J. Physiol. 208: 436-450, 1965.

Parer JT. *The effect of acute maternal hypoxia on fetal oxygenation and the umbilical circulation in the sheep.* Eur. J. Obstet. Gynecol. Reprod. Biol. 10: 125-136, 1980.

Parkes MJ, Moor PJ, Moore DR, Fisk NM, and Hanson MA. *Behavioral changes in fetal sheep caused by vibroacoustic stimulation: The effects of cochlear ablation.* Am. J. Obstet. Gynecol. 164: 1336-1343, 1991.

Patrick J, Natale R, and Richardson B. *Patterns of human fetal breathing activity at 34 to 35 weeks' gestational age.* Am. J. Obstet. Gynecol. 132: 507-513, 1978.

Patrick J, Challis JRG, and Cross J. *Effects of maternal indomethacin administration on fetal breathing movements in sheep.* J. Dev. Physiol. 9: 295-300, 1987.

Phillipson EA, Duffin J, and Cooper JD. *Critical dependence of respiratory rhythmicity on metabolic CO_2 load.* J. Appl. Physiol. 50: 45-54, 1981.

Phillipson EA, and Bowes G. *Control of breathing during sleep.* In: Fishman AP, Cherniack NS, Widdicombe JG Eds. Handbook of Physiology section 3: The Respiratory System, Volume II, part 2. American Physiological Society, Bethesda, U.S.A., 1986, p 649-689.

Power GG, Kawamura T, Dale PS, Schroder H, Gilbert RD. *Temperature responses following ventilation of the fetal sheep in utero.* J. Dev. Physiol. 8: 477-484, 1986.

Power GG, and Longo LD. *Placental O_2 transfer and fetal consumption at varying fetal arterial PO_2.* Fed. Proc. 34: 451, 1975.

Prechtl HFR, Akiyama Y, Zinkin P, and Grant DK. *Polygraphic studies of the full-term newborn:I. Technical aspects and qualitative analysis.* In: R. Mackeith, Bax Eds. Studies in Infancy: clinics in developmental medicine, Heinemann Medical Books, London, U.K., 1968, 27: 1-21.

Quilligan EJ, Clewlow F, Johnston BM, and Walker DW. *Effect of 5-hydroxytryptophan on electrocortical activity and breathing movements of fetal sheep.* Am. J. Obstet. Gynecol. 141: 271-275, 1981.

Reid DL, Jensen A, Phernetton TM, and Rankin JHG. *Relationship between plasma catecholamine levels and electrocortical state in the mature fetal lambs.* J. Dev. Physiol. 13: 75-79, 1990.

Richardson B, Hohimer AR, Mueggler P, and Bissonnette J. *Effects of glucose concentration on fetal breathing movements and electrocortical activity in fetal lambs.* Am. J. Obstet. Gynecol. 142: 678-683, 1982.

Richardson B, Natale R, and Patrick J. *Human fetal breathing activity during electively induced labor at term.* Am. J. Obstet. Gynecol. 133: 247-255, 1979.

Richardson BS, Patrick JE, and Abdul-Jabbar A. *Cerebral oxidative metabolism in the fetal lamb; relationship to electrocortical state.* Am. J. Obstet. Gynecol. 153: 426-431, 1985.

Rigatto H, Blanco CE, and Walker DW. *The response to stimulation of hindlimb nerves in fetal sheep, in utero, during the different phases of elecrocortical activity.* J. Dev. Physiol. 4: 175-185, 1982.

Rigatto H, Moore M, and Cates D. *Fetal breathing and behavior measured through a double-wall plexiglas window in sheep.* J. Appl. Physiol. 61: 160-164, 1986.

Rigatto H, Lee D, Davi M, Moore M, Rigatto E, and Cates D. *Effect of increased arterial CO_2 on fetal breathing and behavior in sheep.* J. Appl. Physiol. 64: 982-987, 1988.

Ritchie JWK, and Lakhani K. *Fetal breathing movements in response to maternal inhalation of 5% carbon dioxide.* Am. J. Obstet. Gynecol. 136: 386-388, 1980.

Rosenberg AA. *Response of the cerebral circulation to profound hypocarbia in neonatal lambs.* Stroke 19: 1365-1370, 1988.

Rosenberg AA, Jones MD, jr., Traystman RJ, Simmons MA, and Molteni RA. *Response of cerebral blood flow to changes in PCO_2 in fetal, newborn, and adult sheep.* Am. J. Physiol. 242: H862-H866, 1982.

Ruckebusch Y, Gaujoux M, and Eghbali B. *Sleep cycles and kinesis in the foetal lamb.* Electroencephalogr. Clin. Neurophysiol. 42: 226-237, 1977.

Rurak DW, and Gruber NC. *Increased oxygen consumption associated with breathing activity in fetal lambs.* J. Appl. Physiol. 54: 701-707, 1983.

Sack J, Beaudry M, DeLamater PV, Oh W, and Fisher DA. *Umbilical cord cutting triggers hypoertriiodothyoninemia and nonshivering thermogenesis in the newborn lamb.* Pediatr. Res. 10: 169-175, 1976.

Santiago TV, and Edelman NH. *Opoids and breathing.* J. Appl. Physiol. 59: 1675-1685, 1985.

Scarpelli EM, Condorelli S, and Cosmi EV. *Cutaneous stimulation and generation of breathing in the fetus.* Pediatr. Res. 11: 24-28, 1977.

Sheldon MI, and Green JF. *Evidence of pulmonary CO_2 chemosensitivity: effects on ventilation.* J. Appl. Physiol. 52: 1192-1197, 1982.

Sherman DJ, Ross MG, Day L, Humme J, and Ervin MG. *Fetal swallowing: response to graded maternal hypoxemia.* J. Appl. Physiol. 71: 1856-1861, 1991.

Sidi D, Kuipers JRG, Heymann MA, and Rudolph AM. *Effects of ambient temperature on oxygen consumption and the circulation in newborn lambs at rest and during hypoxemia.* Pediatr. Res. 17: 254-258, 1983.

Siesjo BK, and Ingvar M. *Ventilation and brain metabolism.* Handbook of Physiology. Section 3: the Respiratory System, Volume II, Control of breathing, part 1. American Physiological Society, Bethesda, U.S.A., 1986, p 141-161.

Sival DA, Visser GHA and Prechtl HFR. *Fetal breathing movements are not a good indicator of lung development after premature rupture of membranes and oligohydramnios - a preliminary study.* Early Hum. Dev. 28: 133-143, 1992.

Slegel P, Kitagawa H, and Maguire MH. *Determination of adenosine in fetal perfusates of human placental cotyledons using fluorescence derivatization and reversed-phase high-performance liquid chromotography.* Anal. Biochem. 171: 124-134, 1988.

Sue-Tang A, Brooks AN, Hooper S, White S, Jacobs R, Bocking AD, and Challis JRG. *Increased circulating corticotrophin-releasing hormone (CRH) occurs during fetal hypoxemia with reduced utero-placental blood flow (UBF), but not as a result of maternal hypoxemia.* Meeting of Society of Gynecologic Investigation, St. Louis, MO, Abstract 319, 1990.

Szeto HH. *Spectral edge frequency as a simple quantitative measure of maturation of electrocortical activity.* Pediatr. Res. 27: 289-292, 1990.

Szeto HH, Cheng PY, Decena JA, Wu D-L, and Dwyer G. *Developmental changes in continuity and stability of breathing in the fetal lamb.* Am. J. Physiol. 262: R452-R458, 1992.

Szeto HH, Vo TDH, Dwyer G, Dogromajian ME, Cox MJ, and Senger G. *The ontogeny of fetal lamb electrocortical: a power spectral analysis.* Am. J. Obstet. Gynecol. 153: 462-466, 1985.

Teitel DF, Iwamoto HS, and Rudolph AM. *Changes in the pulmonary circulation during birth-related events.* Pediatr. Res. 27: 372-378, 1990.

Tiktinsky MH, Hasan SU, Rigaux A, Bishop B, and Morin III FC. *The effect of oxygenation on breathing movements in the fetal lamb.* Pediatr. Res. 31: Abstract 1932, 1992.

Tiktinsky MH, Hasan SU, Rigaux A, Bishop B, and Morin III FC. *Hyperbaric oxygenation increases arousal and breathing movements in fetal lambs.* J. Appl. Physiol. 77: 902-911, 1994.

Towell ME, Johnson J, Smedstad K, Andrew M, and Vu T-L. *Fetal blood and tissue PO_2 during maternal oxygen breathing.* J. Dev. Physiol. 6: 177-185, 1984.

Visser GHA, Poelman-Weesjes G, Cohen TMN, Bekedam DJ. *Fetal behavior at 30 to 32 weeks of gestation.* Pediatr. Res. 22: 655-658, 1987.

Vries de JIP, Visser GHA, and Prechtl HFR. *The emergence of fetal behaviour.* 1. Qualitative aspects. Early Hum. Develop. 7: 301-322, 1982.

Vyas H, Milner AD, and Hopkin IE. *Intrathoracic pressure and volume changes during the spontaneous onset of respiration in babies born by cesarean section and by vaginal delivery.* J. Pediatr. 99: 787-791, 1981.

Walker AM, Oakes GK, Ehrenkranz R, McLaughlin M, and Chez RA. *Effects of hypercapnia on uterine and umbilical circulations in conscious pregnant sheep.* J. Appl. Physiol. 41: 727-733, 1976.

Walker D. *No effect of prostaglandin synthesis inhibition on muscle reflexes in fetal lambs.* Am. J. Physiol. 258: R1213-R1216, 1990.

Walker DW and Davies AN. *Effects of hyperthermia on fetal breathing movements.* J. Dev. Physiol. 8: 485- 497, 1986.

Wallen LD, Murai DT, Clyman RI, Lee CH, Mauray FE, and Kitterman JA. *Regulation of breathing movements in fetal sheep by prostaglandin E2.* J. Appl. Physiol. 60: 526-531, 1986.

Wallen LD, Murai DT, Clyman RI, Lee CH, Mauray FE, and Kitterman JA. *Effects of meclofenamate on breathing movements in fetal sheep before delivery.* J. Appl. Physiol. 64: 759-766, 1988.

Wanatabe T, Kumar P, and Hanson MA. *Effects of warm environmental temperature on the gain of the respiratory chemoflex in the kitten.* J. Physiol. 459: 336P, 1993.

Wardlaw SL, Stark RI, Daniel S, and Frantz AG. *Effects of hypoxia on beta-endorphin and beta-lipotropin release in fetal, newborn, and maternal sheep.* Endocrinology 108: 1710-1715, 1981.

Weering van HK, Wladimiroff JW, and Roodenburg PJ. *Effect of changes in maternal blood gases on fetal breathing movements.* Contrib. Gynecol. Obstet. 6: 88-91, 1979.

Wigglesworth JS, and Desai R. *Effects on lung growth of cervical cord section in the rabbit fetus.* Early Hum. Dev. 3: 51-65, 1979.

Wilkening RB, and Meschia G. *Fetal oxygen uptake, oxygenation, and acid-base balance as a function of uterine blood flow.* Am. J. Physiol. 244: H749-H755, 1983.

Whitehouse MW, and Haslam JM. *Ability of some antirheumatic drugs to uncouple oxidative phosphorylation.* Nature 196:1323-1324, 1962.

Woudstra BR, Aarnoudse JG, de Wolf BTHM, Zijlstra WG. *Nuchal muscle activity at different levels of hypoxemia in fetal sheep.* Am. J. Obstet. Gynecol. 162: 559-564, 1990.

Summary

Breathing movements are periodically present in utero and must become continuous after birth. Mechanisms involved in the control of breathing in utero and involved in the establishment of continuous breathing at birth were of our interest. All fetuses used in this thesis were instrumented at 128-132 days gestation for recording fetal behaviour and for later connection to an ECMO system to change fetal blood gases, and temperature. In order to observe the mechanism involved at the moment of birth some fetuses were instrumented with a cord occluder (chapter 7, 8). The following questions were the subject of our investigation.

1. ***Are breathing movements in utero dependent on the level of $PaCO_2$? (chapter 4)***

Breathing movements are only present during LV ECoG associated with eye movements. HV ECoG is associated with nuchal muscle activity and there are no breathing movements present. Therefore, breathing movements have been suggested to be part of the expression of fetal behaviour. Our first objective was to study the mechanism involved in determining the presence of breathing activity in utero by investigating whether the incidence of fetal breathing movements could be affected by the level of $PaCO_2$. During mild hypocapnia the overall incidence of breathing movements, the incidence of breathing movements during LV ECoG and the mean duration of periods of breathing decreased significantly. Fetal ECoG activity showed normal cycling during the periods of mild hypocapnia and the mean duration of LV ECoG periods did not change. During mild hypocapnia, rapid eye movements (n=3) remained associated with LV ECoG and nuchal muscle activity with HV ECoG. These results suggest that the presence of breathing movements in fetal life is not only dependent on the behavioral state but also on the level of fetal $PaCO_2$.

2. ***Does fetal breathing activity respond to increased levels of CO_2? Does the association of hypercapnia and cooling play a role in the initiation and maintenance of continuous breathing? (chapter 5)***

Secondly, we investigated whether the stimulatory response of hypercapnia on fetal breathing is already present in utero and whether

increased afferent input produced by cooling might change the sensitivity for CO_2 overriding the central inhibition during HV ECoG. This could shed some light on the mechanisms involved in the initiation of continuous breathing at birth. During fetal hypercapnia frequency, amplitude and incidence of fetal breathing movements during LV ECoG increased significantly compared to isocapnic control on ECMO but it remained absent during HV ECoG. During hypercapnia associated with central cooling there were similar changes in fetal breathing movements during LV ECoG, however, in 4 out of 7 fetuses fetal breathing movements continued throughout HV ECoG. Hypercapnia associated with central cooling can thus override the inhibitory effects of HV ECoG on fetal breathing movements. This may suggest that cooling produced changes in CO_2 sensitivity probably by increasing afferent input to the central nervous system.

3. ***Are the inhibitory effects on fetal breathing during fetal hypoxemia an indirect effect due to the release or production of mediators from the maternal side of the placenta or the ewe? (chapter 6)***

Another interesting phenomenon is the observation that fetal hypoxemia inhibits fetal breathing and behavioral activity. The mechanism responsible for this is still under discussion. We examined the possible role of maternal or placental substances released or triggered during hypoxemia which could produce fetal inhibition. There was a decrease in incidence in breathing activity to 21.4 ± 3.5%, however there was no change in length of breathing periods, only in 7 experiments breathing activity stopped within 7 minutes. Fetal ECoG activity, nuchal muscle activity, rapid eye movements, blood pressure and heart rate were present as normal. We conclude that the normal inhibition of fetal activity during hypoxemia does not seem to be mediated by release of factors from the maternal side of the placenta or the ewe.

4. ***Does the exclusion of the umbilical circulation and therefore placental modulators play a role in the initiation of breathing at birth? Does a rise in $PaCO_2$ and changes in temperature play a role during this transition? (chapter 7 & 8)***

Our final objective was to study the possibility that substances produced by the placenta could control breathing activity and the initiation of continuous breathing activity after birth. It has been speculated that the disappearance of some theoretically substances produced by the placenta could play a role in the complicated change from periodic to continuous breathing at birth. Breathing movements which occurred after cord occlusion were always associated with LV ECoG. Our results do not support the hypothesis that the initiation of breathing within 5 minutes of birth is dependent on an inhibitory factor of placental origin since breathing activity was not initiated whenever $PaCO_2$ remained constant. Whenever $PaCO_2$ increased, breathing activity was present. These data suggest that a rise in $PaCO_2$ is crucial for the presence of breathing at

birth. Further, we studied the effect of $PaCO_2$ and temperature in the initiation and maintenance of continuous breathing at birth. After delivering the fetuses in a warm saline bath breathing movements were periodically present. Only after 36-192 min breathing activity became continuously present in all animals (pH 7.20 ± 0.04, $PaCO_2$ 7.35 ± 0.16 kPa and PaO_2 12.78 ± 2.51 kPa when core temperature had fallen by a mean of 1.2 °C). Neonatal breathing activity always stopped by decreasing $PaCO_2$. We conclude that the maintenance of fetal $PaCO_2$ and temperature after cord occlusion delays the establishment of continuous breathing at birth. The level of $PaCO_2$ is crucial in the maintenance of breathing activity.

Conclusions

1. The presence of breathing movements in fetal life is not only dependent on the behavioral state and determined by the level of fetal $PaCO_2$.

2. During fetal hypercapnia frequency, amplitude and incidence of fetal breathing movements during LV ECoG increased significantly compared to isocapnic control on ECMO but it remained absent during HV ECoG.

3. Hypercapnia associated with central cooling can override the inhibitory effects of HV ECoG on fetal breathing movements.

4. The inhibition of fetal activity during maternal hypoxemia does not seem to be mediated by release of factors from the maternal side of the placenta or the ewe.

5. The initiation of continuous breathing at birth is not dependent on an inhibitory factor of placental origin.

6. Maintenance of fetal $PaCO_2$ and temperature after cord occlusion delays the establishment of continuous breathing.

7. The level of $PaCO_2$ is important in the maintenance of breathing activity in the first few hours of life.

Samenvatting

De ademhalingsbewegingen van een foetus zijn periodiek aanwezig, terwijl deze na de geboorte continue aanwezig moeten zijn. In dit proefschrift wordt beschreven welke mechanismen betrokken zijn bij de ademhaling in utero en tevens welke mechanismen een rol spelen bij de overgang van periodiek naar continue ademhaling na de geboorte. Deze mechanismen zijn bestudeerd aan de hand van een onderzoeksopstelling bij foetale lammeren. Bij een zwangerschapsduur van 128-132 dagen (voldragen zwangerschap 145-146 dagen) werden foetale lammeren geïnstrumenteerd voor registratie van electrocorticale activiteit en electromyografische activiteit van diafragma, oogspieren en nekspieren. Verder werden twee catheters ingebracht (a. carotis en v. jugularis) voor connectie aan een extra corporaal membraan oxygenatie (ECMO) systeem. Met gebruik van dit ECMO systeem konden foetale bloedgassen en foetale temperatuur veranderd worden. De invloeden van variaties in bloedgaswaarden en temperatuur van de foetus op de ademhalingsbewegingen werden bestudeerd. Bij enkele foetale lammeren werd een navelstreng occluder aangebracht om eventuele placentaire factoren die van invloed zouden kunnen zijn van de periodieke naar continue ademhaling na de geboorte te kunnen bestuderen.

Aan de hand van dit onderzoeksmodel werden de volgende vragen bestudeerd:

1. ***Is de aanwezigheid van foetale ademhalingsbewegingen afhankelijk van de arteriële pCO_2? (hoofdstuk 4)***

Bij de observatie van de foetale lammeren blijkt dat ademhalingsbewegingen in utero alleen aanwezig zijn in combinatie met laag voltage hersenactiviteit geassocieerd met de aanwezigheid van oogbewegingen (REM-slaap). Tijdens perioden van hoog voltage hersenactiviteit (non-REM slaap) bestaat er een duidelijke associatie met nekspieractiviteit maar vinden er geen ademhalingsbwegingen plaats. In de literatuur wordt daarom gesuggereerd dat de ademhalingsbewegingen een vorm van gedragsuitdrukking zijn, daar deze alleen tijdens REM slaap optreden en niet tijdens non-REM slaap. Om het effect te meten van de invloed van pCO_2 op de ademhalingsbewegingen werd de arteriële pCO_2 gevarieerd tussen normale en matige verlaagde waarden, en werden de ademhalingsbewegingen, oogbewegingen, nekspieractiviteit en hersen-

activiteit geregistreerd. Tijdens de milde hypocapnie (verlaagde pCO_2) veranderde het gedrag van de foetale lammeren niet, de electrocorticale activiteit vertoonde een normale cyclus van REM slaap en non-REM slaap, de gemiddelde lengte van periode van REM slaap nam niet af. De oogbewegingen bleven aanwezig tijdens de REM slaap en de nekspier activiteit bleef geassocieerd met non-REM slaap. De mogelijkheid om tijdens de REM slaap te ademen bleef hetzelfde. Echter, de incidentie van de ademhalingsbewegingen daalde zowel gedurende de totale tijd en als tijdens de REM slaap. Verder, nam de gemiddelde duur van de lengte van de periode van de ademhalingsbewegingen significant af. Deze resultaten suggereren dat ademhalingsbewegingen niet alleen een gedragsuitdrukking zijn maar dat de aanwezigheid van de foetale ademhalingsbewegingen ook bepaald wordt door de $PaCO_2$.

2. ***Worden de foetale ademhalingsbewegingen gestimuleerd door hypercapnie? Spelen hypercapnie in combinatie met centrale afkoeling een rol in de initiatie van de continu aanwezige ademhaling na de geboorte? (hoofdstuk 5)***

Tijdens experimenten met arteriele hypercapnie (verhoogde pCO_2) bij de foetus namen zowel de incidentie, de frequentie als de amplitude van de ademhalingsbewegingen toe in vergelijking met metingen die verricht werden tijdens normocapnie (normale pCO_2). De ademhalingsactiviteit bleef echter een periodiek patroon houden. Deze respons van de ademhaling op hypercapnie blijkt al vroeg in de zwangerschap aanwezig te zijn. Het is beschreven dat de ademhaling continu aanwezig is indien de foetus perifeer afgekoeld wordt. Het is niet bekend of centrale afkoeling een toegenomen afferente input produceert en de gevoeligheid voor CO_2 verandert en op deze wijze de inhibitie van de ademhaling tijdens de non-REM slaap doorbreekt. Dit mechanisme zou echter een belangrijke rol kunnen spelen bij het starten van de continue ademhaling ten tijde van de geboorte. Tijdens hypercapnie in combinatie met centrale afkoeling namen de frequentie, incidentie en amplitude toe. Echter, in 4 van de 7 experimenten waren de ademhalingsbewegingen continu aanwezig, ook tijdens de non-REM slaap. Hypercapnie en centrale afkoeling doorbreken de inhibitie tijdens non-REM slaap. Deze observatie suggereert dat centrale afkoeling veranderingen in de CO_2 sensitiviteit veroorzaakt, hoogst waarschijnlijk door een verhoogde afferente input.

3. ***Wordt de inhibitoire respons op de foetale ademhaling tijdens hypoxemie veroorzaakt door een indirect effect: namelijk het vrijkomen van mediatoren geproduceerd door de moeder of de moederlijke kant van de placenta? (hoofdstuk 6)***

Tijdens foetale hypoxemie worden de foetale ademhaling, de oogbewegingen, de nekspier activiteit geinhibeerd. Het mechanisme voor deze inhibitie is niet bekend. Er is beschreven dat tijdens hypoxemie moederlijke en placentaire hormonen vrijkomen (of worden geproduceerd) die een rol spelen in deze inhibitoire reactie. Tijdens maternale

hypoxemie en foetale normoxie was er een afname van de incidentie van de foetale ademhalingsbewegingen. Er was echter geen verandering in de lengte van de periode van de ademhalingsbewegingen en in 7 van de 14 experimenten stopten de ademhalingsbewegingen binnen 7 minuten. Ook was er geen verandering meetbaar in de foetale hersenactivitieit, nekspier activiteit, oogbewegingen, bloeddruk en hartslag. Onze conclusie is dat de reguliere inhibitie van de ademhalingsbewegingen tijdens hypoxemie niet veroorzaakt wordt door het vrijkomen van mediatoren geproduceerd door de moeder of de moederlijke kant van de placenta.

4. ***Spelen bij de initiatie van de continue ademhaling na de geboorte een daling c.q. het wegvallen van door de placenta geproduceerde mediatoren, een toename van de arteriele pCO_2 en een verandering in de omgevingstemperatuur een rol? (hoofdstuk 7 & 8)***

Er werd onderzocht of hormonen c.q. modulatoren geproduceerd door de placenta invloed kunnen hebben op de initiatie van de ademhaling. Hiervoor werd tijdens experimenten de navelstreng geoccludeerd zodat de umbilicale circulatie werd uitgeschakeld en de invloed van de eventuele placentaire hormonen c.q. modulatoren kon worden uitgesloten. Na de navelstreng occlusie kwamen alleen ademhalingsbewegingen voor tijdens laag voltage hersenactiviteit. Deze resultaten steunen niet de hypothese dat de initiatie van de ademhaling na navelstreng occlusie afhankelijk is van de daling van de plasmaspiegels van inhibitoire hormonen/modulatoren geproduceerd door de placenta wanneer de $PaCO_2$ gelijk blijft. Wanneer echter de arteriële pCO_2 steeg was er wel ademhalingsactiviteit aanwezig. Deze bevinding ondersteunt de stelling dat een stijging in de arteriële pCO_2 cruciaal is voor het optreden van ademhalingsbewegingen.

Het effect van $PaCO_2$ en perifere afkoeling werd onderzocht in lammeren geboren in een warm bad gevuld met fysiologisch zout. De temperatuur van het fysiologische zout daalde geleidelijk. Ademhalingsbewegingen waren periodiek aanwezig. Pas dertig minuten tot drie na de geboorte waren de ademhalingsbewegingen in alle lammeren continue aanwezig. Tot die tijd bleven ze periodiek aanwezig. Neonatale ademhalingsactiviteit (continu aanwezige ademhaling) stopte als de arteriële pCO_2 weer werd verlaagd. Uit deze resultaten kan geconcludeerd worden dat het handhaven van de $PaCO_2$ en temperatuur na navelstreng occlusie de initiatie van de continu aanwezige ademhaling tijdens de overgang van het intra-uterien naar het extra-uterien milieu vertraagt. Het handhaven van de $PaCO_2$ is cruciaal voor het aanwezig zijn van de continue ademhaling.

Conclusies

1. De aanwezigheid van foetale ademhalingsbewegingen in utero is niet alleen een uitdrukking van gedrag maar is ook afhankelijk van de arteriële pCO_2.

2. In utero is er een toename van incidentie, frequentie en amplitude van de ademhalingsbewegingen tijdens hypercapnie. Echter, deze ademhalingsbewegingen blijven een periodiek patroon houden.

3. Tijdens hypercapnie in combinatie met centrale afkoeling kan foetale ademhaling continu aanwezig zijn, ook tijdens non-REM slaap.

4. De inhibitie van de foetale activiteit tijdens hypoxemie wordt niet veroorzaakt door het vrijkomen van mediatoren geproduceerd door de moeder of door de moederlijke kant van de placenta.

5. De initiatie van de continue ademhaling na de geboorte is niet afhankelijk van een daling c.q. het wegvallen van door de placenta geproduceerde mediatoren.

6. Normocapnie en normothermie na navelstreng occlusie vertragen de initiatie van de continue ademhaling.

7. Na de geboorte is het niveau van de $PaCO_2$ belangrijk voor de aanwezigheid van ademhalingsactiviteit.

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Curriculum Vitae

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